NEDERL. AKADEMIE VAN WETENSCHAPPEN

PROCEEDINGS

VOLUME XLV

No. 8

President: J. VAN DER HOEVE
Secretary: M. W. WOERDEMAN

CONTENTS

Allgemeine Pathologie. — *Die Bedeutung von hydro- und aërotherapeutischen und einigen anderen Tatsachen für unsere Einsicht in Erkältung und verwandte Störungen.* Von N. PH. TENDELOO.

(Communicated at the meeting of September 26, 1942.)

Die damals täglich gebrauchten Begriffe Konstitution und Erkältung wurden in den letzten 40 Jahren des vorigen Jahrhunderts durch die sich raschentwickelnde Bakteriologie allmählich zurückgedrängt, bis man durch fortgesetzte Wahrnehmung des kranken Menschen, zum Teil ergänzt durch Autopsie und annähernd geprüft auf die klinischen und autoptischen Möglichkeiten an bestimmten homoiothermen Säugetieren, zur heutigen Schlussfolgerung kam, dass in bestimmten Fällen infektiöse Veränderungen infolge von Abkühlung bestimmten Grades und bestimmter Dauer beim Menschen möglich sind. Tödliche Erfrierung mit unaufhaltsamem Sinken der Körpertemperatur und mit Blutdissolution oder ohne dieselbe kommt nicht weiter in Betracht.

Bei all diesen und künftig zu erwähnenden Untersuchungen kommt es jedoch nicht allein auf den Grad (Reizstärke) und die Dauer der Abkühlung, sondern ausserdem auf die persönliche Empfindlichkeit (Reizbarkeit) des Individuums an, wobei Person und Persönlichkeit als Spezies des Genus Individuum betrachtet werden. Die Konstitution ist in der normalen persönlichen Empfindlichkeit für Erkältung sehr wahrscheinlich die Hauptsache, die aber bloss vorübergehend durch eine Störung, oder dauerhaft durch organische Veränderung überstimmt werden kann. So kann z.B. akuter Schnupfen einem Sänger das Singen kurze Zeit verbieten; es kann aber Narbenbildung in den Stimmbändern oder deren Umgebung und dergl. das Singen für immer unmöglich machen. Es kann ein Schnelläufer durch Ueberanstrengung in ungeeignete „Kondition" geraten aber sich erholen, während eine unheilbare Herzkrankheit das Schnellaufen für immer unmöglich macht.

Die persönliche Empfindlichkeit ist eine zusammengesetzte Grösse, deren zusammensetzende Eigenschaften und Verrichtungen sich ein- oder gegenseitig beeinflussen dürften, etwa wie die Atome oder Atomgruppen in einem grossen Molekül. Man kennt diese zusammengesetzte Grösse noch keineswegs ausreichend, sie erscheint jedoch jedesmal unabweisbar, wenn die Wirkung eines Reizes bei den Individuen einer durch diese Reizstärke und Reizdauer bedingte Gruppe unverkennbare Unterschiede aufweist. Weiter aber auch dann, wenn dieselbe Person ab und zu Verschiedenheiten ihrer Empfindlichkeit zeigt, z.B. durch Ermüdung, wobei man jedoch immer die übrigen Eigenschaften und Verrichtungen, auch „Konditionen", und die Möglichkeit, dass noch unbekannte Faktoren mitwirken könnten, im Auge behalten soll.

In diesem Jahrhundert haben die Wahrnehmungen späterer Forscher von Reaktionen des Menschen auf thermische Reize, ausgehend von den grundlegenden Untersuchungen von WINTERNITZ über Hydrotherapie, stark zugenommen und dann sind sie auf die Aërotherapie ausgedehnt worden. Und zwar nicht nur in Anstalten, sondern auch ohne Anstalt am eigenen Leibe oder an Tieren.

Die Ergebnisse der dabei festgestellten Tatsachen dürfen die gleiche Wissenschaftlichkeit (Wahrscheinlichkeit) beanspruchen wie andere als wissenschaftlich betrachtete Forschungen, wenn man mit wissenschaftlich meint: das sachliche allseitig vollständig erstrebend.

Dies gilt nicht am wenigsten für das Gebiet der Abhärtung, d.h. der Versuche den Menschen unempfindlich zu machen gegen Abkühlung durch Wasser oder Luft, indem man eine rasche vollständige „Reaktion" erstrebt als Zeichen der Unempfindlichkeit, welche im Nu erscheinen soll, während Ermüdung nur geringen Einfluss haben dürfte. Bei Kranken ist die Wirkung jedoch nicht immer gleich wahrscheinlich infolge krankhafter Verwicklungen. Man übertreibe aber, beiläufig bemerkt, auch bei Gesunden, die Abhärtung nicht; sie fordert Zeit, also Geduld; namentlich intellektuelle Arbeiter sollen sich davor

hüten, schon deshalb, weil stark abgehärtete Menschen, die im Winter in ein nicht einmal stark geheiztes Zimmer kommen, leicht Kongestionen nach dem Kopfe bekommen, welche ihrer Besinnung kaum zugute kommen dürften.

Man darf nunmehr die „Reaktion" auf Abkühlung durch Wasser oder Luft gelungen nennen, wenn das abgekühlte grosse Hautgebiet warm und trocken geworden oder geblieben ist, also ohne merkbare Schweissbildung bezw. ohne verstärkte Schweissbildung, mit der Empfindung einer allgemeinen Erfrischung und manchmal mit Förderung eines zuvor trägen Stuhlganges.

Ein solches ausgedehntes abgekühltes Hautgebiet bekommt einen grösseren Gehalt an arteriellem Blut von grösserer Volumengeschwindigkeit mit Erhaltung, wenn nicht Vermehrung der arteriellen Wandspannung. Man darf diese Reaktion deshalb arterielle Hautreaktion nennen. Sie heisst allgemein, falls sie in einem ausgedehnten Hautgebiet erscheint, sonst beschränkt, obgleich die Grenzen keine scharfen sind und Kombinationen vorkommen.

Ausserdem darf man annehmen, dass die arterielle Hautreaktion auf Abkühlung durch Wasser oder Luft im allgemeinen um so rascher und stärker erscheint, je nachdem die abgekühlte Haut zuvor wärmer, das abkühlende Wasser oder die Luft kälter, und die Dauer der Abkühlung kürzer ist.

Später hat man eine andere Kombination von Erwärmung (Erhitzung) und Abkühlung mit oder ohne Reiben der Haut angefangen und fortgesetzt, wenn man eine kalte oder ermüdete Haut oder die Haut bestimmter Kranken zu einer baldigen arteriellen Hautreaktion zwingen will (heiss-kalt): man erhitzt zunächst die Haut in einem heissen Vollbad von 39—40° während 5 Minuten oder unter einem Regenbad (Regendusche) der gleichen Temperatur und Dauer, sofort gefolgt von einem möglichst kalten Regenbad, höchstens 1 Minute, oder von einem kalten Wasserstrahl aus einer Giesskanne, morgens oder abends, je nach Umständen, und gegebenenfalls mit Abänderungen, aber auf jeden Fall mit rascher Abtrocknung mit einem trocknen groben, nicht erwärmten Badehandtuch oder Frottierhandschuhen (nicht erwärmt, wenn nicht zum Trocknen, weil die erhitzte Haut venös hyperämisch werden könnte) und dann: sofort in bereit gelegte, nicht erhitzte Leibwäsche und dann *entweder* in einem Bettuch und ausreichenden wollenen Decken (ohne Schwitzkur!) ins Bett, *oder* sich rasch kleiden, nach raschem Frühstück marschieren, draussen bei geeignetem Wetter, sonst in einem geschützten Raum, bis die arterielle Hautreaktion vollkommen entwickelt ist; denn zunächst muss die Abkühlung durch das kalte Wasser hinausgearbeitet werden, was etwa 40 Minuten oder länger dauern mag. Im Bett sind in bestimmten Fällen Muskelanstrengungen und tiefe Atmungen am Platze.

Was ist die wirksamste Temperatur des kalten Wassers aus der Regendusche oder aus der Giesskanne? Nach vorgenommenen Versuchen hängt dies bei gleicher persönlicher Empfindlichkeit von A, von den anderen Temperaturen ab. Ist z.B. im Sommer die Temperatur des Badezimmers 25°, die des Schlafzimmers 26°, und fängt der Bader A an, seine vielleicht etwas feuchte Haut mit einem Frottierhandschuh oder mit einem weniger groben Tuch abzutrocknen und reibt er sich dann ohne besondere Kraft die Rumpfhaut mit dem Frottierhandtuch, getaucht in das Leitungswasser, dessen Temperatur in einem Waschbecken auf 17° bestimmt ist, welche er dann mit einem groben Badehandtuch kräftig und rasch abtrocknet, so erscheint im Nu eine starke arterielle Hautreaktion noch ehe die Rumpfhaut abgetrocknet wurde, sie war aber nicht sicher vollkommen. Je niedriger die Temperaturen sind, um so langsamer, bei gleichem Vorgehen. So auch in der Haut der Beine. Trotzdem wäre die Körperbewegung zur Vervollkommnung der arteriellen Hautreaktion durchzuführen. Später, als A stärker war, sind obige Befunde bestätigt worden.

Sowohl die Kleider als auch die Leibwäsche sollen mit Hinsicht auf die Wärmestrahlung, Wärmeleitung, Wärmekonvektion und Natur des Stoffes Poren von bestimmtem Durchmesser haben, damit die Kleidung mitsamt der Leibwäsche weder durch zu festes Gewebe noch durch zu lockeres Gefüge abkühlt statt dass sie erwärmt. Muss man sich einmal gegen einen scharfen Wind schützen, weil es keine andere Möglichkeit gibt, so umhülle

man sich unter dem Ueberzieher mit einem Tierfell oder mit zähem Packpapier und auch den Kopf entsprechend.

Bei Abkühlung durch Luftbäder, wobei der Hirnschädel (das Cranium) nicht den Sonnenstrahlen ausgesetzt sein darf, kann man die arterielle Hautreaktion fördern, indem man ab und zu umhermarschiert, barfuss, wohl über Kiesel als mechanischen Reiz oder nicht, unter tiefen Atmungen und gymnastischen Bewegungen mit den Armen.

Bei diesen Untersuchungen hängt, wie immer, der Wärmegrad der Haut ab; einerseits von der Menge arteriellen Blutes mit einer Temperatur von ungefähr 37°, welche in einer bestimmten Zeit aus tieferen Teilen, vor allem aus Muskeln und Drüsen, den Hautgefässen zugeführt wird, andrerseits von der Abkühlung dieses Blutes an der Hautoberfläche durch Wärmestrahlung, Wärmeleitung und Wärmekonvektion, wobei der Wärmegrad, Wassergehalt und Bewegung der umgebenden Luft, die Natur (s. oben) der Kleider, Leibwäsche und Decken massgebend sind.

Eine feuchte Haut soll abgetrocknet werden, damit nicht durch Verdampfung in gewissem Umfang thermische Verklammung erfolge. Schwitzen ist nur gestattet bei einer Schwitzkur und in Fällen worin Verdampfung des Schweisses einem Anstieg der Bluttemperatur vorbeugt, wie z.B. bei Heizern in Dampfschiffen im Roten Meer.

Allgemeine (thermische) *Verklammung* mit krankhafter oder wenigstens unverkennbar abnormer Folge betrachte man als Erkältung. Es gibt eine allgemeine, eine beschränkte Verklammung und eine Kombination.

Verklammung ist der arteriellen Hautreaktion vollständig entgegengesetzt: sie ist die Folge einer Verengerung durch Abkühlung nicht allein der gleichen Hautschlagadern mitsamt ihren Haargefässen, deren Erweiterung die arterielle Hautreaktion bedeutet, aber ausserdem noch von Verengerung venöser Blutgefässe in diesem Hautgebiet, mit Abnahme der gesamten Menge, der Volumengeschwindigkeit und des Sauerstoffgehalts dieses Blutes. Die Blutfarbe nähert sich infolgedessen der Farbe des sauerstofflosen Hämoglobins, so dass die Haut cyanotisch wird im Gegensatz zur hellroten Farbe des sauerstoffreichen Bluts bei der arteriellen Hautreaktion. Beide Farbenveränderungen sind um so leichter erkennbar, je nachdem die Oberhaut dünner ist und die Farbe des Hämoglobins in ihren Blutkapillaren folglich klarer durchschimmert.

Ferner geht Verklammung wohl oder nicht mit Frösteln, Gänsehaut, Zähneklappern, Zuckungen willkürlicher Muskeln, Empfindung von Unbehagen und Unfähigkeit einher, alles in verschiedener Stärke bis zum Verlust des Bewusstseins und schliesslich gar des Lebens. In Schwimmanstalten in freier Luft kann man die leichteren Störungen bei ruhenden Schwimmern manchmal sehen, bei Badern im offenen Meer ausserdem nicht selten ernstere Ereignisse, sogar plötzlichen Tod. Diese ernsteren Fälle sind jedoch nicht einheitlichen Ursprungs: es gibt Bader mit einem Herzfehler, die die Abkühlung mit Wellenschlag nicht vertragen, während dieser kräftigere Menschen erwärmt; andere haben einen vollen Magen und ersticken, falls sie erbrechen, im Erbrochenen, das in die Luftwege angesaugt wird; wieder andere mit einem Loch in einem Trommelfell werden wahrscheinlich schwindelig und ertrinken. Verklammung ist offenbar auch etwas anderes als die soeben erwähnte venöse Hyperämie durch Erhitzung.

Nach vielfach bestätigter Wahrnehmung am Menschen fördern im allgemeinen Körperbewegung und Sonne die arterielle Hautreaktion, Ruhe und Schatten hingegen führen vielmehr zu Verklammung, während Zug die Abhärtung (Unempfindlichkeit) auf die Probe stellt.

Durch Verklammung wird das Blut aus der abgekühlten Haut in tiefere Teile hineingezwängt. Die Haut kann schon bei einsetzender Verklammung in hohem Grade erblassen, so dass Leute mit grosser Empfindlichkeit die Finger durch Waschen in kaltem Wasser leichenblass („tote Finger") machen, woraus ein Nadelstich kein Blut herauszubringen vermag.

Durch Verschiebung von Blut aus der verklammten Haut in tiefere Körperteile werden diese blutreicher, wahrscheinlich z.B. die Lunge, Luftröhre Bronchi und Bronchiolen. Von dem Sauerstoffgehalt und der Stromgeschwindigkeit des Blutes in diesen Teilen weiss man

aber recht wenig. Die Lufterneuerung in den Lungen und damit der Sauerstoffgehalt des Blutes in diesen Teilen kann aber nicht allein für die verschiedenen in Betracht kommenden Bakterien eine verschiedene Bedeutung haben, sie dürfte auch nicht gleichgültig sein für den Zustand und die übrigen Verrichtungen des Lungengewebes selbst. Für den Gaswechsel in den Lungen ist die lineare Stromgeschwindigkeit ihres Blutes massgebend. Der zunehmende Blutgehalt dieser Teile dürfte nach sonstigen allgemein-pathologischen Erfahrungen Entzündung fördern können.

Jetzt folgt die Mitteilung zweier Wahrnehmungen von Verklammung.

Ein bejahrter Mann nahm schon viele Jahre täglich, so auch im Dezember 1941, ein heiss-kaltes Regensturzbad, mitunter statt der Dusche kaltes Wasser aus der Giesskanne, kleidete sich und frühstückte rasch. Ab und zu verzögerte er dann aber durch besondere Umstände die erforderliche sofortige Körperbewegung, zweimal sogar um etwa 2 Stunden, und lief dann etwa 25 Minuten in einem scharfen Wind draussen. Er setzte sich das erste Mal in einem mässig geheizten Zimmer hin, fiel aber bald bewusstlos zu Boden, kam aber offenbar bald zu sich, bleich mit einer oberflächlichen Schramme auf der Stirn.

Nach einigen Tagen setzte er sich um 12.30 an den Kaffeetisch hin, mit unvollständiger arterieller Hautreaktion, und kündigte bald einen bevorstehenden Bewusstseinsverlust an. Er kam zu sich, liegend auf einem Diwan neben dem Kaffeetisch unter einer wollenen Decke, und sah einen ihm sehr wohl bekannten, aber erst nach einiger Zeit erkannten Arzt über sich gebeugt. Dieser hatte ihm soeben 1 ccm Cardiazol unter die Haut des rechten Arms eingespritzt, weil er den Radialispuls nicht fühlen konnte. Der Arzt bestimmte dann in der üblichen unblutigen Weise den Blutdruck in der rechten Arteria brachialis auf 145/175, während er Gesicht und Ohren wachsbleich (also leichenblass) nannte. Als er nach einiger Zeit den Radialispuls fühlte, verabschiedete er sich, während er die wachsbleiche Hautfarbe noch sah.

Ein Kollaps infolge ungenügender Herzwirkung kommt kaum in Betracht, weil der arterielle Blutdruck dabei gerade tief sinkt, während er hier als hoch festgestellt wurde. Sollte man diese Blutdruckerhöhung dem Cardiazol zuschreiben wollen, so blieben das Ausbleiben des Radialispulses und die grosse Hartnäckigkeit der wachsbleichen Farbe von Gesicht und Ohren ungeklärt. Es vermag jedoch Verklammung des ausgedehnten abgekühlten Hautgebiets nicht allein ohne weiteres die wachsbleiche Hautfarbe und als Folge dieser erheblichen Verengerung eines grossen Arteriengebiets die Blutdruckerhöhung in der Art. brachialis verständlich zu machen, sondern auch das Ausbleiben des Radialispulses als Folge von Verklammung, indem diese oberflächliche Schlagader ebenfalls verklammte, ähnlich wie in „toten Fingern" (s. oben).

Wodurch die wachsbleiche Farbe länger aushielt, wäre noch zu erforschen. Vielleicht durch die grössere Tiefe der Verklammung. Auf ihre grosse Tiefe in der Gegend des Hirnschädels weisen die Bewusstseinstörungen durch Verklammung auch der Hirnrinde hin. Die persönliche Empfindlichkeit dieses Mannes für thermische Reize hatte in letzter Zeit zugenommen seit der Zusammenwirkung von zwei strengen Wintern, wogegen er sich nicht immer ausreichend zu schützen schien, mit Distribution, und den unzureichenden Körperbewegungen zur Ausbildung der arteriellen Hautreaktion.

Verklammung mit ähnlichen Erscheinungen hat sich aber auch ohne Bad nach Abkühlung verschiedener Art von gewisser Dauer und Stärke im Zusammenhang mit der persönlichen Empfindlichkeit, in verschiedenen Graden als möglich erwiesen. Den Erfahrungen bei Schwimmern in freier Luft in Schwimmanstalten oder im Meer, schliessen sich die bei gewissen Schlittschuhläufern an, die sogar mit arterieller Hautreaktion die Schlittschuhe abschnallen und sich, unausreichend gekleidet, nach Sonnenuntergang einige Zeit einem scharfen Winde aussetzten ohne ausreichende Körperbewegung: es kann Verklammung zu Schnupfen, Tracheitis, Bronchitis, Bronchiolitis, Lungenentzündung oder zum Aufflackern einer noch nicht erkannten oder klinisch geheilt gewähnten Lungentuberkulose führen, alles infektiöse Veränderungen.

Etwas anders ist der baldige Tod nach Schlittschuhfahren oder nach einem anstrengenden Ausflug indem ein durch Atrophie, Verengerung von Kranzschlagadern oder sonstige

Veränderungen abgeschwächtes Herz einer so hohen Anforderung nicht gewachsen war.

Gibt es Erkältung ohne Verklammung? Sofern Redner bekannt ist, nicht. Man kennt aber eine Gruppe von Menschen mit mehr oder weniger verwandten (analogen) Erscheinungen, mit Störungen des Bewusstseins und der Herzwirkung infolge von Schreckern oder Entsetzen, oder mit gestörter Herzwirkung toxischen Ursprungs, wie z.B. Angina pectoris infolge chronischer Nikotinvergiftung. Vielleicht wäre Verengerung von Blutgefässen dabei mit Verengerung infolge von Verklammung gleichzustellen.

Man hat in Holland den Einfluss der verschiedenen Winde auf den Menschen annähernd zu bestimmen gesucht, zunächst durch Prüfung mehrerer Windfahnen in verschiedener Höhe in der unmittelbaren Nähe; dabei beachtete man aber die Möglichkeit, dass schwache Winde nicht jede Windfahne zu drehen vermögen, auch die Möglichkeit, dass ein Wind in der Nähe eines Gebäudes, um die Ecke, durch Richtungsänderung oder durch Erweiterung einer Strasse, Wirbel bildet usw. Das Urteil wird durch Aehnliches erschwert.

Aus diesen Untersuchungen geht aber hervor, dass man meist bei bestimmten Windrichtungen, gewisser Windstärke und gewissen Temperaturen der Luft eine rauhe, scharfe, sogar eisige Kälte sowohl im Hause als draussen empfinden kann, die durch Mark und Bein dringt, sogar im Sommer; durch Absperrung von Spalten in Fenstern und an Türrändern vermag man diese Kälte auszuschliessen. Diese Kälte macht sich in Holland vor allem bemerkbar bei Nordwest-, Nord-, Nordost- und Ostwinden; die Empfindung der Kälte hört aber meist auf, sobald der Wind Südost- oder Süd wird. Südwestwinde sind meist wieder etwas kälter, während der Westwind in der Regel eine entspannende wohltätige, obwohl manchmal eine erschlaffende Wirkung hat. Ob dem westlichen Sturmwind an unserer Nordseeküste eine andere Bedeutung zukommt, ist Redner nicht bekannt.

Folgender Versuch möge obiges einigermassen verdeutlichen. Ein Zimmer von gleicher Länge und Breite (je 400 cm) und 330 cm Höhe gibt in seiner nördlichen Wand, nicht gerade in der Mitte, durch eine nach aussen sich öffnende Flügeltür, deren jede Tür 83 cm breit und 227 cm hoch ist, Zugang zu einem Balkon 265 cm breit (West-Ost) und 168 cm tief (Nord-Süd). Nach dem Sonnenstand um 12 Uhr liegt der Balkon nicht genau gegen Norden, der Untershcied ist für den Versuch aber von keiner Bedeutung so dass wir ihn zur Vereinfachung vernachlässigen. (Fig. 1.)

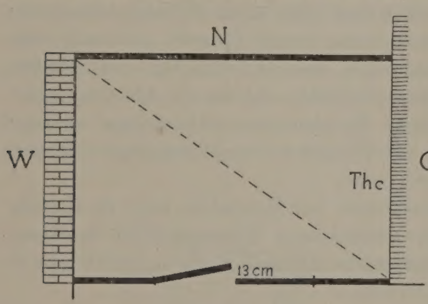


Fig. 1.

Die Westseite des Balkons wird durch eine Mauer abgegrenzt die hart an der Zimmermauer 222 cm hoch und im nördlichen Fünftel 178 cm hoch ist. Annähernd die westliche Hälfte des Balkons liegt immer im Schatten. Die Ostseite wird abgegrenzt durch eine Hausmauer 223 cm breit, somit zu 55 cm ausserhalb des Balkons, hoch bis zur Dachrinne. In der Mitte des Balkons ist ein Thermometer Th aufgehängt, welches die Sonne im Sommer nachmittags bestrahlt so dass er bis 30° und höher ansteigt. Trotzdem gelingt es dann, wenn die Ostbalkontür mittels einer Espagnolette geschlossen und die Westbalkontür auf eine Spalte von 13 cm nach Osten mittels eines Hakens verklammert ist, die Zimmertemperatur annähernd auf 18° zu halten: das Zimmerthermometer hängt in der Mitte der südlichen Zimmerwand, zwei Zimmertüren sind zugfrei geschlossen; der Rollvorhang ist auf 100 cm herabgelassen, während einige cm unter diesem Vorhang eine filzige Zugschutzdecke (tochtdeken) senkrecht bis auf den Boden reicht.

Steckt man nun eine Hand mit dem Rücken gen Osten durch den freien dreieckigen Teil der Spalte, so fühlt man mit diesem Handrücken (im Schatten!) bloss eine schwache Himmelstrahlung,, aber am Handteller, sogar bei Südwestwind, einen kalten Luftstrom, der in das Zimmer hereindringt. Sobald die Sonne untergeht, sinkt die Säule im Th bald auf etwa 17° oder niedriger, wenn nämlich der Wind nicht Südost, Süd oder West ist. In diesen drei Fällen hat man im Sommer gewöhnlich Sommerwetter; es steigt dann auch

die Zimmertemperatur bis etwa 30° nachts an. Bei anderen Winden möge der zweckmässig gekleidete nicht überempfindliche Spaziergänger das Wetter, besonders in der Sonne, loben, im Hause ist es dann manchmal frostig ohne Absperrung der Spalten. Wer eine Markise oder sonstigen Sonnenschirm bei geöffnetem Fenster oder Tür gebraucht, möge sich nicht wundern, wenn nach einigen Stunden oder am nächsten Morgen eine wunde oder schmerzhafte Empfindung im Rachen oder unter dem Brustbein (mögliches Zeichen von Tracheitis) erscheint, vielleicht mit einer Spur schleimigen Auswurfs, der zunächst an der Schleimhaut haftet, aber nachher gelockert wird entweder durch Blutentziehung infolge einer kräftigen arteriellen Hautreaktion, welche damit die Schleimhaut durch Oberflächenzunahme dehnt, oder indem eine kräftige Einatmung oder Husten dies bewirkt.

Im Gegensatz zu den oben erwähnten infektiösen Veränderungen in tieferen Teilen nach Verklammung ist die *heilkräftige* Wirkung einer Reihe arterieller Hautreaktionen auf Bronch(iol)itis mit oder ohne Asthma-anfällen unverkennbar. Es hat sich sogar ereignet, dass schon die erste arterielle Hautreaktion einer solchen Reihe bei einem Kind sofort einen nächtlichen Anfall beendete, wie sie während 2 Jahre regelmässig erschienen waren. Auch Asthma im Anschluss an Pseudokrupp erfährt diese heilende Wirkung. Diese Fälle wurden, sofern Redner bekannt ist, behandelt mit heiss-kalten Bädern, wie oben angegeben wurde. Wahrscheinlich entziehen die kräftigen arteriellen Hautreaktionen den tieferen Teilen, Blut, wodurch die Bronchiolen weiter wurden und die Atmung freier. Durch wiederholte Blutentziehungen kann nach anderen pathologischen Erfahrungen (s. oben) die Entzündung der Bronchi(olen) allmählich ausheilen und ihre normale Konstitution nach klinischer Beurteilung wiederhergestellt werden, solange nicht zu alte Veränderungen oder etwas anderes dem im Wege steht, wie z.B. Idiosynkrasie.

So kann man auch dem Kopfe Blut entziehen, indem man sich bei gewisser Empfindlichkeit und zu warmer Luft oder zu warmer Kleidung oder bei Ermüdung die Weste aufknöpft, so dass die Bauchhaut durch Abkühlung arterielle Hautreaktion bekommt; oder indem man in Rückenlage die beiden Beine ohne Schuhe oder sogar barfuss senkrecht in die Höhe steckt und, abhängig von der Lufttemperatur, einige Minuten senkrecht hält. Die Unterschenkel werden dann abgekühlt, aber wenn man in Strümpfen und Schuhen umhergeht, bekommen sie arterielle Hautreaktion, welche wie durch Abkühlung der Bauchhaut dem Hirn Blut entzieht mit allgemeiner Erholung und Erfrischung, während Schwerhörigkeit, welche wenigstens zum Teil auf Hyperämie des Gehörorgans beruht, verbessert.

Auch die *beschränkte* Abkühlung von gewisser Dauer und Reizstärke kann im Zusammenhang mit der persönlichen Empfindlichkeit verschiedene Veränderungen bewirken. Diese Empfindlichkeit kann sich allmählich sogar kaum merkbar ändern, ähnlich wie in den im vorigen mitgeteilten Beobachtungen.

Als Gegenstück zur soeben genannten Blutentziehung aus dem Gehirn hat man haufig Schnupfen Bronchiolitis usw. mit oder ohne Asthma nach Abkühlung der Unterschenkel beobachtet, entweder durch Zug oder durch ein kaltes Fussbad am Strand, namentlich bei scharfem Wetter.

Zu den leichtesten Veränderungen gehören die „rheumatischen" Nerven- und Muskelschmerzen des Gesichts und der Kopfhaut, Lumbago (Hexenschuss), Ischias, Schiefhals, Facialislähmung, entstanden durch Stehen, Sitzen oder Liegen in einem Luftzug in der Nähe einer nicht abgesperrten Spalte eines Fensters oder einer Tür, ferner auch bei Schwimmern in freier Luft oder im Meer. Auch ein mitunter oft wiederholter Schnupfen, Tracheitis, Bronchi(oli)tis. Hier kommen aber ohne scharfe Grenzen Uebergänge zu schwereren Fällen vor. Infektion ist nicht nachgewiesen ober annehmlich gemacht, im Gegensatz zum Rheumatismus articulorum acutus.

Während die alltäglichen Frostbeulen (Perniones) durch Stromverlangsamung oder Stillstand in einem beschränkten Gefässgebiet der Finger, zu oberflächlichen und hieraus entstehenden tiefen Geschwüren führen können, so gibt es auch eine scheinbar unmittelbare Nekrose (Tod eines Körperteils) von Fingerteilen infolge von Abkühlung, wie es scheint nicht viel beobachtet. Wieder etwas anderes ist die oberflächliche, nur selten

tiefere Erfrierung von Ohren und Nase in freier Luft bei starkem Frost. Auch zu unterscheiden ist die bloss scheinbare Erfrierung eines Fusses oder Unterschenkels bei Soldaten im Laufgraben (pied de tranchée), meist durch mässige, feuchte, lang andauernde Kälte.

Die hier gemeinte Nekrose wurde beobachtet bei einem älteren Mann, der mit Winterübeln und Distributionsfolgen auf einem Diwan ruhte: sofort nach schnellem Sinken der Zimmertemperatur von 16° auf ungefähr 8° infolge von Stockung der heizenden Gaszufuhr. Bei ihm wurden bald zwei Nekroseformen wahrnehmbar, die sich auf die beiden Ring- und fünften Finger und den linken Mittelfinger beschränkten, während die anderen Finger viel geringere und der rechte Zeige- und Mittelfinger mehr als ein Jahr ältere, nicht ausgeheilte Veränderungen mit Oedem, Par- und Hypästhesien aufwiesen, häufig verschlimmert durch Stösse und vielfaches Schreiben. Fragt man, ob die persönliche Empfindlichkeit oder die Schädigung mehr auf die 2 Ring- und die 2 fünften Finger lastet, so sind diese normalen Finger die schwächsten mit anscheinend zarterer Haut, während die beiden rechten Finger während des Schreibens einigem Druck ausgesetzt sind. Ausserdem waren diese 4 Finger schon einige Wochen vor der Nekrose mehr oder weniger im palmaren Teil (Greiffläche) ihrer Endglieder rötlich geschwollen, der linke Mittelfinger ähnlich, aber nur in der Kuppe, d.h. einem Abschnitt so gross wie etwa die Hälfte einer grauen Erbse. Im übrigen erscheint der normale linke Mittelfinger schwächer und zarter als der normale rechte.

Die Nekrose der beiden linken Finger erscheint oberflächlicher und beschränkter als die der beiden gleichnamigen rechten Finger. An jenen linken Fingerendgliedern bildete sich zugleich eine Blase, jedoch nicht mit einer weichen sondern mit einer pergamentartigen etwas durchscheinenden Wand ähnlich, aber nicht so durchscheinend wie die von Zuckerbäckern zum Schutz ihres Gebäcks gegen Fliegen und Speichel gebrauchte Membrane. Das aus Oberhaut entstandene Fell war tote Oberhaut, durch welche vergrösserte und etwas rötliche Papillen und Papillengruppen des Corium durchschimmerten, während vereinzelt solche Knötchen auch proximal des freien Nagelrandes den Nagel etwas hervorwölbten. Die Flüssigkeit in diesen Blasen wurde binnen zwei oder drei Tagen resorbiert, und die Wand abgeschnitten mit nachfolgender Ausheilung nach etlichen Wochen.

Die beiden gleichnamigen rechten Finger wurden von einer tieferen und ausgedehnteren Nekrose befallen: es erschien an der lateralen Fläche des rechten Ringfingers (auf der Seite des fünften Fingers) in etwas mehr als den zwei distalen Fingergliedern die Haut glatt, gespannt und von bräunlicher Farbe, welche proximal am hellsten, etwas gelblich war, zum Nagel hin aber allmählich dunkler wurde (Fig. 2). Bei möglichst schwacher Berührung zeigte sich diese in verschiedenen Schattierungen bräunliche Haut als unempfindlich, bei leichtem Druck wurde jedoch ein glühender Schmerz, ohne Druck ein brennender Schmerz angegeben. Die bräunliche Haut sah aus wie durch einen glühenden Körper gesengt. Die Haut des lateralen Nagelwalls (des Hautsaumes lateral vom Nagel) war vernichtet und hart daneben fehlte in der Tiefe im lateralen rechten Rande des Wurzels (Lunula) des Nagels ein Stück, alsob es die Hälfte eines herausgeschlagenen Punzierlochs von etwa 2 mm Durchschnitt wäre, von einem keineswegs klaren Mechanismus. Ganz in der Nähe fand sich tief im proximalen Teil des Nagelwalls eine Schrunde (Rhagade) mit roten Rändern, deren Berührung sofort den glühenden Schmerz erweckte; dies beweist offenbar nicht, dass ein schmerzerregender Stoff an Ort und Stelle vorhanden wäre; es können die Gewebsveränderungen dafür verantwortlich sein, worauf das sofortige fast vollkommene Aufhören der Schmerzhaftigkeit nach dem Abschneiden eines lateralen distalen Nagelteils hindeutet (s. Fig. 2), wodurch wahrscheinlich eine gewisse Entspannung folgte. Das baldige Aufhören des Schmerzes durch Untertauchung des Fingers in Wasser von Zimmertemperatur oder nach Auflegen von kühlendem reinem Lanolinum, obwohl der Schmerz nachher abgeschwächt mitunter zurückkehrte, wäre auch anders zu deuten.

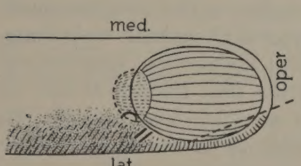


Fig. 2.

Es vermehrte sich das von der Oberhaut gebildete sogen. Sohlenhorn etwas, es liess sich aber absatzweise leicht entfernen. Vermehrung dieses Horns ist nichts besonderes: thermische Reize erregen überhaupt bei ausreichender Wiederholung Hornbildung auch an der flachen Haut, wie z.B. an der Oberfläche des im übrigen wenig veränderten linken Zeigefingers bei diesem Mann, wodurch die Hautoberfläche das wohlbekannte schmutzigbraune Aussehen mit feinen Stacheln gewann. Das Sohlenhorn wird gebildet durch die Falten Epidermis auf Corium unter dem freien Nagelrand; diese Epidermis hängt gar nicht mit dem Hyponychium zusammen, und die „Verhornung" des Nagels unterscheidet sich wesentlich von der der Oberhaut.

Die Veränderungen des rechten fünften Fingers sind denen des Ringfingers ähnlich, aber in verjüngtem Massstabe. Nach der Ausheilung war die Oberhaut dieser beiden Finger zart.

Der linke Mittelfinger bekam zunächst eine kleine pergamentartige Blase mit durchschimmernden Hautpapillen wie der linke Ring- und Oberfinger (s. oben). Es kamen jedoch an der Fingerkuppe zwei sichelförmige Schrunden hinzu, welche sich nahezu senkrecht halbierten, so dass 4 Kreissektoren entstanden, welche der Ausheilung hartnäckig Widerstand leisteten, vielleicht noch hartnäckiger als die berüchtigte Stichwunde der alten dreikantigen Bajonette, indem die Zipfel der geschrumpften Sektoren keinen Anschluss fanden, aber schliesslich blieb eine empfindliche Narbe (Fig. 3).

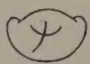


Fig. 3.

Zugleich mit dem Anfang dieser Veränderungen entstand an der linken Zehe des Mannes eine typische juckende Frost- oder Blutbeule (Pernio), wie er noch nie gehabt hatte. Sie schwand binnen zwei Stunden unter dem Einfluss feuchtwarmer Luft.

Es erhebt sich die Frage, ob die arterielle Hautreaktion nur Begleiterscheinung ist einer umfassenderen, das Wesen einschliessenden Veränderung aller in Betracht kommenden Zellen, auch ausserhalb der Blutgefässe. Man vermag diese Frage nicht, auch nicht annähernd, zu beantworten ehe wir dazu ausreichend von den möglichen tieferen Veränderungen wissen. Hiervon sind wir jedoch noch weit entfernt. Auch die bisher nachgewiesenen Stoffwechselstörungen, sofern Redner diese kennt, reichen dazu nicht aus. Wir sind nicht einmal berechtigt es unwahrscheinlich zu nennen, dass sie Folge der Aenderung des Blutstroms sind. Aehnliches gilt für die Verklammung.

Gibt es auch Verklammung *nicht* thermischen Ursprungs? Vielleicht gibt es eine solche unter dem Einfluss nicht-thermischer Strahlen, aber Redner könnte keinen ausreichend wahrscheinlichen Hinweis nennen. Sämtliche derartige Wirkungen, sofern nicht thermische atmosphärische Faktoren in Betracht kämen, weisen im Gegenteil Hyperämie, wahrscheinlich entzündlicher, deshalb jedoch nicht infektiöser Natur, weil Entzündung, Infektion und auch Fieber oft zusammentreffen, jedoch verschiedene Begriffe sind. Handelt es sich um einen Fall ohne nachweisbare oder wenigstens annehmlich gemachte Verklammung, wie z.B. um eine Pseudokrupp nach Einwirkung eines heissen trocknen Ostwindes im Sommer, so ist von einer arteriellen Verengerung eines ausgedehnten Hautgebietes mit Verengerung von Venen oder ohne solche, wie bei Verklammung durch Abkühlung, bisher nichts beobachtet worden. Es könnte aber in irgend einer Weise ohne Abkühlung Verengerung der Schlagadern eines Hautgebiets erfolgt sein. Wir müssen weitere Forschungsergebnisse abwarten. Die Atmosphäre birgt viele Möglichkeiten durch Wirkung von elektrischen und anderen Strahlen in sich. Aber auch trockne heisse Luft im Hause durch Heizung macht obengenannte Schleimhäute blutreich.

LITERATUR.

WINTERNITZ, Die Hydrotherapie auf physiol. und klin. Grundlage 1890, und Dr. B. BUXBAUM, Lehrb. d. Hydrother., Leipzig 1900, bes. S. 1—70; E. VAN EVERDINGEN, Samenvatting Congres Geographie Amsterdam 1938; N. PH. TENDELOO, Studien u. d. Ursachen der Lungenkrkh., Wiesbaden 1902, Ss. 252—255, 2. A. München 1931, Alg. Ziekt. 1938 pp. 44 (venen), 66—70, 76—82, 141—154, 194—196, 325—329, 675.

Mathematics. — *Beweis dass der Begriff der Menge höherer Ordnung nicht als Grundbegriff der intuitionistischen Mathematik in Betracht kommt.* Von Prof. L. E. J. BROUWER.

(Communicated at the meeting of September 26, 1942.)

In meiner Note: „*Zum freien Werden von Mengen und Funktionen*" [1]) wurde das Verfahren M_σ betrachtet, durch welches der Fundamentalreihe F' der in einer beliebig ein für allemal bestimmten Weise abgezählten endlichen Wahlfolgen von Nummern eineindeutig und ähnlich ein beliebiges Element σ der Menge [2]) M zugeordnet wird. Wir wollen dieses Verfahren M_σ als *Menge zweiter Ordnung* und die solchermassen den unbegrenzten Wahlfolgen von Nummern zugeordneten Folgen von Zeichenreihen als *die Elemente der Menge zweiter Ordnung* M_σ bezeichnen.

Die in meiner zitierten Note ausgesprochene Behauptung, dass M_σ eine Teilspezies einer aus M herleitbaren Menge M_1 darstellt, und dass die Vereinigung aller von M erzeugten M_σ mit diesem M_1 identisch ist, soll im Folgenden bewiesen werden. Wir befassen uns zunächst mit der Konstruktion der Menge M_1.

Sei $\alpha_1\, \alpha_2 \ldots \alpha_m$ eine endliche Wahlfolge von Nummern. Die Rangnummer derselben in der Fundamentalreihe F' bezeichnen wir mit $\varrho(\alpha_1\, \alpha_2 \ldots \alpha_m)$ und das Maximum der Nummern $\varrho\,(\alpha_1)$, $\varrho\,(\alpha_1\, \alpha_2)$, $\ldots$ $\varrho\,(\alpha_1\, \alpha_2 \ldots \alpha_m)$ mit $\zeta\,(\alpha_1\, \alpha_2 \ldots \alpha_m)$.

Die Kombination einer beliebigen Nummer α_1 mit $\varrho\,(\alpha_1)$ beliebigen Nummern β_1, β_2, $\ldots$ $\beta_{\varrho(\alpha_1)}$ nennen wir eine K-Kombination. Die K-Kombinationen zählen wir durch eine Fundamentalreihe F ab. Diejenige K-Kombination, welche in F die Rangnummer ν_1 erhält, bezeichnen wir mit K_{ν_1}.

Für gegebenes ν_1, mithin ebenfalls gegebenes α_1, und beliebiges α_2 nennen wir im Falle $\zeta\,(\alpha_1\, \alpha_2) = \zeta\,(\alpha_1)$ die Nummer α_2 und im Falle $\zeta\,(\alpha_1\, \alpha_2) > \zeta\,(\alpha_1)$ die Kombination von α_2 mit $\zeta\,(\alpha_1\, \alpha_2) - \zeta(\alpha_1)$ beliebigen Nummern $\beta_{\zeta(\alpha_1)+1}, \ldots \beta_{\zeta(\alpha_1 \alpha_2)}$ eine K_{ν_1}-Kombination. Für jedes ν_1 zählen wir die K_{ν_1}-Kombinationen durch eine Fundamentalreihe F_{ν_1} ab. Diejenige K_{ν_1}-Kombination, welche in F_{ν_1} die Rangnummer ν_2 erhält, bezeichnen wir mit $K_{\nu_1 \nu_2}$.

Für gegebene ν_1 und ν_2, mithin ebenfalls gegebene α_1 und α_2, und beliebiges α_3 nennen wir im Falle $\zeta\,(\alpha_1\, \alpha_2\, \alpha_3) = \zeta\,(\alpha_1\, \alpha_2)$ die Nummer α_3

---

1) Proc. Ned. Akad. v. Wetensch. Amsterdam, **45**, 322 (1942).

2) Der Einfachheit halber beschränken wir uns in dieser Note auf solche Mengen, in deren Erzeugungsprozess weder Hemmung noch Beendigung auftritt. Diese Beschränkung ist unwesentlich.

und im Falle $\zeta(\alpha_1\alpha_2\alpha_3) > \zeta(\alpha_1\alpha_2)$ die Kombination von α_3 mit $\zeta(\alpha_1\alpha_2\alpha_3) - \zeta(\alpha_1\alpha_2)$ beliebigen Nummern $\beta_{\zeta(\alpha_1\alpha_2)+1}, \dots \beta_{\zeta(\alpha_1\alpha_2\alpha_3)}$ eine $K_{\nu_1\nu_2}$-Kombination. Für jedes Nummernpaar ν_1, ν_2 zählen wir die $K_{\nu_1\nu_2}$-Kombinationen durch eine Fundamentalreihe $F_{\nu_1\nu_2}$ ab. Diejenige $K_{\nu_1\nu_2}$-Kombination, welche in $F_{\nu_1\nu_2}$ die Rangnummer ν_3 erhält, bezeichnen wir mit $K_{\nu_1\nu_2\nu_3}$.

In dieser Weise fortfahrend, definieren wir für jede natürliche Zahl s die $K_{\nu_1\nu_2\dots\nu_s}$. Dabei tragen wir Sorge, von vornherein ein Gesetz zu bestimmen, durch welches sämtliche jedesmal die $K_{\nu_1\nu_2\dots\nu_s}$ abzählende Fundamentalreihen $F_{\nu_1\nu_2\dots\nu_s}$ ein für allemal festgelegt werden.

Die Konstruktion von M_1 wird nunmehr vollzogen, indem wir jedesmal der endlichen Wahlfolge $\nu_1\nu_2\dots\nu_s$ diejenige Zeichenreihe zuordnen, welche für die entsprechenden Nummern $\alpha_1, \alpha_2, \dots \alpha_s$, $\beta_1, \beta_2, \dots \beta_{\varrho(\alpha_1\alpha_2\dots\alpha_s)}$ in M der endlichen Wahlfolge $\beta_1\beta_2\dots\beta_{\varrho(\alpha_1\alpha_2\dots\alpha_s)}$ zugeordnet ist.

Sei σ das von der unendlichen Wahlfolge $\gamma_1\gamma_2\gamma_3\dots$ erzeugte Element von M. *Alsdann ist die Menge zweiter Ordnung M_σ mit einer Teilspezies ${}_\sigma M_1$ von M_1 identisch.* Dieses ${}_\sigma M_1$ entsteht, wenn in M_1 für jedes s nur solche ν_s gewählt werden dürfen, denen $K_{\nu_1\nu_2\dots\nu_{s-1}}$-Kombinationen entsprechen, in welchen jedes β_τ dem den gleichen Index tragenden γ_τ gleich ist.

Sei umgekehrt e ein beliebiges Element von M_1. Alsdann bestimmt die e in M_1 erzeugende unbegrenzte Wahlfolge $\nu_1\nu_2\nu_3\dots$ nach der obigen Definition der ν_s gleichzeitig eine unbegrenzte Nummernfolge $\beta_1\beta_2\beta_3\dots$, welche ihrerseits in M das Element $\sigma(e)$ erzeugt. Das zugehörige $M_{\sigma(e)}$ enthält e als Element. *Mithin ist M_1 mit der Vereinigung aller von M erzeugten M_σ identisch.*

Aus dem vorstehenden geht hervor, dass der Begriff der Menge zweiter Ordnung nicht als Grundbegriff der intuitionistischen Mathematik in Betracht kommt.

Um den Begriff der *Menge höherer Ordnung* zu definieren, nehmen wir an dass der Begriff der *Menge n-ter Ordnung* bereits definiert sei und betrachten das Verfahren $(M^{(n)})_\sigma$, durch welches der Fundamentalreihe F' der in einer beliebig ein für allemal bestimmten Weise abgezählten endlichen Wahlfolgen von Nummern eineindeutig und ähnlich ein Element σ der Menge n-ter Ordnung $M^{(n)}$ zugeordnet wird. Dieses Verfahren $(M^{(n)})_\sigma$ bezeichnen wir als *Menge $(n+1)$-ter Ordnung* und die solchermassen den unbegrenzten Wahlfolgen von Nummern zugeordneten Folgen von Zeichenreihen als *die Elemente der Menge $(n+1)$-ter Ordnung* $(M^{(n)})_\sigma$.

Nun ist aber eine Menge zweiter Ordnung M_σ eine Teilspezies einer aus der M_σ zugrunde liegenden Menge M herleitbaren Menge M_1, d.h. ein beliebiges Element π von M_σ ist gleichzeitig Element von M_1. Hieraus folgt,

dass die Menge dritter Ordnung $(M_5)_\pi$ mit der Menge zweiter Ordnung $(M_1)_\pi$, d.h. eine beliebige Menge dritter Ordnung mit einer Menge zweiter Ordnung identisch ist. Und hieraus folgt weiter, dass auch für beliebiges n eine beliebige Menge n-ter Ordnung mit einer Menge zweiter Ordnung identisch ist.

Mithin stellt sich heraus, dass auch der Begriff der Menge höherer Ordnung, im Gegensatz zum Begriff der Spezies höherer Ordnung, nicht als Grundbegriff der intuitionistischen Mathematik in Betracht kommt.

Botany. — *Absorption and transport by the tentacles of Drosera capensis.* II. *The activation of the transport of different substances by oxygen.* By W. H. ARISZ.

(Communicated at the meeting of September 26, 1942.)

§ 1. *Introduction.*

In a previous paper it has been shown that the transport of asparagine by the tentacles of DROSERA is a process dependent on aerobic respiration. The tentacles take up asparagine even from an asparagine solution of very low concentration and transport it through their parenchyma cells to the lamina. This process may take place against a concentration-gradient. There does not seem to exist any fundamental difference between the process of absorption and that of transport of asparagine. In both cases the protoplasm accelerates a transfer of asparagine. Therefore we shall not make a difference between absorption- and transport-processes in this paper. It is essential to know whether this active transport is a special process, only obtaining for asparagine, or whether it also obtains for related substances as amino-acids, and how other organic and inorganic substances are transported. For there are many indications in literature that also other substances are taken up. This was already concluded by DARWIN from the aggregation and granulation caused by various substances in the parenchymacells of the pedicel.

ARISZ and OUDMAN (1937) have corroborated the observation of Miss KOK (1932) and of OUDMAN (1936), viz. that the absorption of caffeine much resembles a process of diffusion. In this communication it will be shown that the absorption of caffeine is not merely a process of diffusion after all, as it proceeds more strongly in the presence of oxygen than without oxygen. This activating influence of oxygen, which is particularly strong for aminoacids and asparagine, is found again in all substances examined in a greater or less degree. This also holds good for some inorganic substances.

The arrangement of the experiments and the method of research has already been described in the preceding publication. In these experiments I again had the valuable assistance of Miss J. VAN WEERDEN and Miss J. VAN DER SCHANS. The phosphate determinations have been made with ammonium-molybdate by using a colorimetric method, after destruction of the leaves with concentrated H_2SO_4 and HNO_3. Though in accuracy this determination is inferior to the Micro-Kjeldahl method, the values for the quantities of phosphates absorbed are sufficiently large for us to draw our conclusions. The accuracy of the experiments is mainly dependent on the uniformity of the plants examined in a certain experiment. This causes much more uniform results in one experiment than in another.

§ 2. *Influence of Oxygen on the absorption and the transport of organic substances.*

The influence of a decrease of the oxygen-pressure on the absorption of asparagine by the tentacles was already discussed in the preceding paper. In the same way the absorption of other organic substances has now been examined. In table I their data have been given. The following substances were examined: urea, methyl-urea, thio-urea, phenyl-urea, glycocoll, α alanine, leucine, phenylalanine, asparagine, urotropine and caffeine. All experiments were continued for 24 hours at a temp. of 25° C. The concentration in which the substances were administered in the agar, amounted to $^1/_{20}$ m in every case.

Various of these substances, as asparagine and the aminoacids cause a curving of the tentacles out of the agarstrips. In experiments with these substances we usually find already after 24 hours the tentacles curved out of the agar and their glands pressed against it. Though they are therefore still in touch with the agar, their uptake is reduced.

In a concentration of $^1/_{20}$ m, urea, thio-urea and caffeine cause no curving of the tentacles. In lower concentrations there may occur a curve in the case of caffeine. As was already shown in the previous communication addition of sucrose to the agar in a concentration of 0.35 m reduces the turgescence of the tentacles so much that an inflection is prevented. This method has also been used in many of these experiments to prevent curving of the tentacles from the agar. In table I the quantity of nitrogen taken up in

TABLE I. Absorption of organic substances in normal and anaerobic conditions. The concentration of all substances is 1/20 m. Duration of absorption 24 hours. 25° C. Sucrose 0.35 m has been added to the agar.

Substance	Nitrogen increase in $^0/_{00}$ of fresh weight		Anaerobic as $^0/_0$ of normal	Mol. w.	Conc. of absorbed substance in leaves in millimol	
	Normal	Anaerobic			Normal	Anaerobic
Urea	0.87	0.21	24 $^0/_0$	60.05	31	7.5
Methyl-urea	0.31	0.13	42 $^0/_0$	74.06	11	4.6
Thio-urea	0.49	0.27	55 $^0/_0$	76.11	17.5	9.6
Phenyl-urea	0.43	0.28	65 $^0/_0$	136.08	15	10
Urotropine	0.69	0.45	65 $^0/_0$	140.13	12	8
Caffeine	0.93	0.64	69 $^0/_0$	194	17	11.4
Glycocoll	1.05	0.03	3 $^0/_0$	75.05	75	2.1
α alanine	0.90	0.04	4 $^0/_0$	89.06	64	2.8
Leucine	0.52	0.—	0 $^0/_0$	131.11	37	0.—
Asparagine	1.15	0.15	12 $^0/_0$	132.08	41	5.4

24 hours has been given, expressed in $^0/_{00}$ of fresh weight of the leaves, in normal circumstances, i.e. in the presence of oxygen in the air and in anaerobic conditions. Moreover the concentration of the absorbed substance in millimol present in the leaf at the end of the experiment has been given in the last two columns. It has been taken for granted that the substance taken up does not undergo any transformation [1]). On comparing these figures one should take into account that the amount of the absorption of a substance may differ a good deal in different experimental series, because plants of various sizes and various ages have been used. The experiments extend over some years and have been made in various times of the year, in consequence of which the condition and the reactivity of the plants have not been the same in all experiments. Besides in experiments in which curving of the tentacles has been prevented by the addition of sucrose to the agar, a comparatively greater quantity is taken up, so that experiments made in this way, are not to be compared with experiments without the addition of sucrose. Of course this does not obtain for experiments with urea, thio-urea and caffeine. As the tentacles do not curve out in these cases, it does not matter whether sucrose has been added or not. If an other concentration than $^1/_{20}$ m had been chosen, the absolute value of the quantities absorbed would have been altered for some substances, for others it would not in the same degree, and owing to this the relative proportion in which the substances are taken up, would have been modified too. It is therefore permitted to compare the values of table I for the absorption of *one and the same* experimental series under normal and under anaerobic conditions, but too great a value should not be attached to the difference in the amount of the uptake of *different* experimental series. Therefore we only produce the figures of the last two columns to give the reader a provisional impression of the relative amount in which various substances containing a different number of nitrogen atoms have been absorbed. From the table it appears that with all substances examined the absorption is

[1]) This presupposition seems permitted, as OUDMAN could not show any protein-sythesis in the leaves after feeding.

diminished by decrease of oxygen. In order to get an impression about the extent to which withdrawal of oxygen affects the process of absorption, the quantity of nitrogen absorbed anaerobically, expressed in percents of that which is absorbed in normal conditions, has been given in the fourth column. This value enables us to compare the influence of oxygen withdrawal on the absorption for various substances. When the values obtained for amino-acids are compared with those obtained for asparagine in the previous paper (ARISZ, I) it appears that these substances behave in the same way and that on removal of oxygen, transport is as good as impossible. In how far a slight transport takes place anaerobically cannot be decided, as the values found are within the limits of error of the determinations. It is surprising that also urea, methyl-urea, thio-urea, phenyl-urea and caffeine are transported in greater quantities in aerobic than in anaerobic conditions. For caffeine we find that anaerobically about 70 % of the quantity absorbed in normal conditions is taken up. So in this case even without the influence of aerobic respiration a rather strong absorption takes place.

From these data the general conclusion must be drawn, that the acceleration caused in the transport by aerobic respiration, is a process which occurs in the transport of all substances examined. As long as only the influence was known which decrease of oxygen-pressure has on the absorption and the transport of asparagine, we might get the impression that there existed a sharp distinction between an active transport in the case of asparagine, which is dependent on aerobic respiration and a passive transport in the case of caffeine which would be based on diffusion. If, however, we consider the results of a decrease of oxygen-pressure on the uptake of different substances, it is clear that such a sharp contrast does not exist between actively and passively transported substances. There is a gradual transition from asparagine and amino-acids to urea, methyl-urea, thio-urea, phenyl-urea and caffeine. The activation cannot but be based on an influence which originates from the protoplasm. As a measure of activation we shall use

$$100\left(1-\frac{\text{anaerobic absorption}}{\text{aerobic absorption}}\right).$$

In table 2 the values for the activation of the various substances examined have been given. The special behaviour of asparagine and the amino-acids is clearly visible, in which transport is activated for nearly 100 %. Next urea, methyl-urea, thio-urea, phenyl-urea, urotropine and caffeine follow, the activation of the transport growing smaller and smaller in this series, until the transport rather resembles an ordinary diffusion-process.

TABLE II. Activation of the transport of different substances. The activation is expressed as $100\left(1-\frac{\text{anaerobic transport}}{\text{aerobic transport}}\right)$.

	Activation		Activation
Glycocoll	97	Methyl-urea	58
Alanine	96	Thio-urea	45
Leucine	100	Phenyl-urea	35
Asparagine	88	Urotropine	35
Urea	76	Caffeine	31

On the basis of the data now obtained the behaviour of caffeine deviates somewhat from what has been found before.

Miss KOK examined the diffusion of caffeine in the pedicel by ascertaining after different periods, in how far granulation had occurred in the vacuole of the cells. She found that the distance covered in the transport is proportional to the square root of the time, as according to STEFAN may be expected with a diffusion process. In her opinion the transport-route for caffeine is mainly the vacuole. The acceleration which according to

the experiments discussed above appears to occur under the influence of respiration in the transport of this substance, must, however, be based on an influence executed by the protoplasm. We shall have to assume that this acceleration affects the transport during the passage of the caffeine through the protoplasm from one vacuole to the adjoining one. Miss KOK states that the resistance offered by the protoplasm to the transport is 160 times larger than that of the vacuole (KOK 1932, p. 104). In her experiments of short duration diffusion must have been relatively strong, whereas the activated transport was relatively slighter than in experiments mentioned here, which lasted 24 hours. In her short experiments Miss KOK found no influence of aether-narcosis, whereas OUDMAN with an experiment that lasted 18 hours, found a feeble inhibition of the transport of caffeine by aether-narcosis. With narcosis the transport amounted to 79.6 % of the normal one. This observation can be understood, now that it has been shown that also in the transport of caffeine protoplasmic activation cooperates.

After all the uptake of caffeine is chiefly a diffusionprocess. From experiments with different concentrations of caffeine it appears that in 24 hours no typical accumulation is found. The difference with the accumulation of asparagine becomes obvious in comparing table III with table IV of the first publication of this series (ARISZ, I).

TABLE III. Absorption of caffeine from different concentrations. 25° C.

m conc. of caffeine in agar	Nitrogen-increase in ‰ of fresh weight of leaves	m conc. of caffeine in leaves	Accumulation-factor
0.05	0.81	0.0145	0.29
0.0125	0.38	0.0068	0.54
0.003125	0.17	0.0030	0.96
0.000781	0.		

The data of tables 1 and 2 give rise to the question whether there is any connection between the nature of the substance and the strength of the transport. For the permeation of substances through the protoplasm into the vacuole general rules have been found concerning the connection between the nature of the organic substances and their power of permeating. So COLLANDER assumes that substances permeate better according as they are more lipoid-soluble, while at the same time the molecular volume has its influence. When we trace whether there is any connection between the lipoid-solubility of the substances and the strength of their transport, we should distinguish between the activated transport in aerobic conditions and the transport by diffusion when oxygen is withdrawn. If the latter was purely a diffusionprocess, influence of lipoid-solubility and molecular volume might be expected. For lipoid-soluble substances permeate rapidly through the protoplasm into the vacuole and diffusion in the vacuole takes place more rapidly than in the protoplasm. The data obtained (table I, last column) do not yet enable us to answer this question. Thio-urea having a greater lipoid-solubility than urea, it might be expected that anaerobically more thio-urea than urea would be taken up. This is indeed the case, but only in a slight degree. Caffeine, of which the lipoid-solubility is great and which gives a precipitation in the vacuole, is transported anaerobically more strongly than urea, which is lipoid-soluble to a less degree and has a much smaller molecule. The transport of caffeine is also stronger than that of thio-urea and urotropine, which have a smaller molecule. The very slight anaerobic transport of asparagine and amino-acids is very likely connected with the dissociation of these substances. On the whole dissociated substances permeate badly. In connection with what has been said above on the comparability of the various experimental series, the data mentioned are insufficient to prove a connection between lipoid-solubility and anaerobic transport.

Let us now consider whether there is any connection between the quantity of substances absorbed under normal, aerobic conditions their lipoid-solubility and their molecular

TABLE IV. Absorption of urea, methyl-urea, thio-urea and phenyl-urea in a concentration of 1/20 m and of 1/80 m. All experiments with addition of sucrose to the agar. Column I contains dates on the molecular refraction, column II gives the oil/water distribution coefficients.

	I	II	Increase of nitrogen in $^0/_{00}$ of fresh weight of leaves	
	Mol. refr.	Oil / Water	24 hours 1/20 m	42 hours 1/80 m
Urea	13.7	0.00015	0.66	0.71
Methyl-urea	18.5	0.00044	0.42	0.18
Thio-urea	20.9	0.00120	0.26	0.14
Phenyl-urea	35.7		0.38	0.23

volume. Table IV and V contain some data which may be mutually compared. Every figure is the average of two or more experiments in which the uptake of the different substances is determined in the same series. In table IV data are given relating to the active absorption of urea, methyl-urea, thio-urea and phenyl-urea. Though in permeation experiments methyl-urea and thio-urea are found to permeate better according to their greater lipoid-solubility, here the active absorption of urea from $^1/_{80}$ m solution is about 4 times as large as that of methyl- and thio-urea.

TABLE V. Absorption of acetamide, lactamide, malonamide, butyramide and succinimid. All experiments with addition of sucrose to the agar. In column I the molecular refraction, in column II the oil/water distribution-coefficients.

	I	II	Increase of nitrogen in $^0/_{00}$ of fresh weight of leaves	
	Mol. refr.	Oil / Water	24 hours 1/20 m	48 hours 1/80 m
Acetamide	14.9	0.00083	0.25	0.17
Lactamide	21.—	0.00058	0.18	0.12
Malonamide	22.9	0.00008	0.28	0.07
Butyramide	24.1	0.00950	0.27	0.12
Succinimid	27.1	0.00490	0.27	0.11

Also in the series with amides (table V) butyramide and malonamide though greatly differing in lipoidsolubility are taken up in the same amount. Moreover the molecular volume of the different amides seems to have no great influence on the active uptake. Here therefore there is certainly no connection in the sense that substances are absorbed better according as they have a smaller molecular volume or are more lipoidsoluble.

On the contrary it appears from the data obtained that as a substance permeates better through the protoplasm it is less activated in the transport (see also table II). It stands more or less to reason that a substance which easily permeates through the protoplasm and arrives in the vacuole is but slightly transported by the plasm.

In the series of the aminoacids (table VI) the strength of the uptake from $^1/_{20}$ m solutions is about the same for glycocoll and alanine being less for leucine and phenyl-alanine. From $^1/_{320}$ m solutions the absorption is much smaller for phenylalanine than for the other aminoacids. As the aminoacids are highly dissociated they cannot easily permeate in the living cell. (SCHÖNFELDER 1930) It is interesting that SCHMENGLER (1933) in experiments with a collodion-membrane obtained a much better permeation of phenylalanine than of aliphatic aminoacids. According to SCHMENGLER the better permeation of this aromatic aminoacid depends on the formation of amphions in a smaller amount by

aromatic than by aliphatic aminoacids (BJERRUM 1923). In the here mentioned experiments with active uptake we see the opposite phenomenon. Phenyl-alanine, that permeates better, is here less strongly absorbed than the aliphatic aminoacids. It is probable that the amphions of the aminoacids are actively taken up. In experiments on the active absorption of asparagine by the leaves of Vallisneria spiralis (ARISZ and VAN DIJK 1939, p. 830) we arrived at the same supposition. When phenyl-alanine forms amphions in a smaller degree its smaller absorption can easily be understood. So we find here the same rule that *substances are more actively absorbed and transported in proportion as they permeate less well through the plasm.*

TABLE VI. Absorption of amino-acids. All experiments with addition of sucrose to the agar.

	Mol. refr.	Increase of nitrogen in $^0/_{00}$ of fresh weight of leaves	
		24 hours 1/20 m	24 hours 1/320 m
Glycocoll	16.4	0.80	0.32
Alanine	21.01	0.70	0.32
Leucine	34.87	0.47	0.30
d Phenyl-alanine	45.2	0.29	0.13

§ 3. *Influence of oxygen on the absorption and the transport of inorganic substances, especially of phosphates.*

SCHMID (1912) and RUSCHMANN (1914) have shown by micro-chemical methods that after feeding with phosphates the presence of phosphoric acid could be shown in the leaf of DROSERA. In addition they could prove the absorption of potassium.

The phosphates appeared to be very suitable for an investigation on absorption, though a few difficulties were encountered. In the first place the determination of the absorbed phosphates was not so accurate as that of nitrogen with the Micro-Kjeldahl method, while the phosphates are less strongly absorbed than the amino-acids. Owing to the mono-potassium phosphate the tentacles curve, so that most of the experiments mentioned in this paper have been made with addition of sucrose to the agar. Di-potassium phosphate does not cause curving of the tentacles. The absorbtion is very slight in this case.

In the quantitative determination of phosphate we encounter the difficulty that the secretion of the tentacles influences the pH of the agar, which factor also affects the dissociation of the phosphates. We shall first discuss the uptake and transport of the KH_2PO_4. Table VII column I gives the concentration of the KH_2PO_4 in the agar; in the second column we find the quantity of phosphate absorbed, calculated as P_2O_5 and in the third column the concentration KH_2PO_4 in the leaf, assuming that the substance

TABLE VII. Accumulation of KH_2PO_4 in leaves of Drosera capensis. In all experiments 0.35 m sucrose is added to the agar. Duration of absorption 24 hours, 25° C.

m conc. of KH_2PO_4 in agar	Increase of P_2O_5 in $^0/_{00}$ of fresh weight of leaves	m conc. of KH_2PO_4 in leaves	Accumulation factor
0.05	0.71	0.01	0.2
0.0125	0.71	0.01	0.8
0.003125	0.72	0.01	3.2
0.000781	0 66	0.0093	11.9
0.000195	0.41	0.0058	29.6

remains unchanged in the leaf and is equally divided over all leaf cells. In the last column the accumulation-factor has been given.

The result of the experimental series is clear. Up to a concentration of about 1/320 m the absorption increases, according as the concentration increases. With a further rise in the concentration of KH_2PO_4 in the agar the absorption does not increase any more and the maximum transport-capacity has been attained. Then the concentration of the phosphate is no more a limiting factor for the strength of the transport, so that the maximum transport strength has been attained for this temperature. The accumulation factor for a concentration of 1/5120 m is about 30. Therefore the uptake of phosphate is just like that of amino-acids and asparagine an accumulation process. In this case we find for the uptake of phosphates almost the same connection between concentration and strength of uptake as in the preceding publication for asparagine. Also for glycocoll (cf. this series No. III) a similar connection has been found.

From the experiments mentioned in table VIII on the influence of removal of oxygen

TABLE VIII. Absorption of phosphates in normal and anaerobic conditions. The concentration of all substances is 1/20 m. 25° C.

Substance	Duration of absorption in hours	Increase of P_2O_5 in ‰ of fresh weight		Anaerobic as % of normal
		Normal	Anaerobic	
KH_2PO_4	24	0.46	0.18	39.1 %
KH_2PO_4	48	1.09	0.15	13.8 %
KH_2PO_4	48	0.57	0.08	14.— %
KH_2PO_4	44	0.21	0.01	

on the uptake of KH_2PO_4 it appears that this process also depends on the oxygen-pressure of the air and therefore in this respect also corresponds with amino-acids. How strong the activation of the transport in this case is, cannot be ascertained with great accuracy. From the figures obtained it appears that the average activation is 78 %. It seems, however, very well possible, that the activation is considerably greater, but the accuracy of the determinations does not allow of our pronouncing an opinion on this. At any rate it appears from this figure that the protoplasm also in the transport of phosphates acts a very important part. All these data indicate that phosphates are transported in essentially the same way as amino-acids and asparagine. In a following publication we shall revert to this.

In table VIII figures have also been given about the uptake of KH_2PO_4 in normal and in anaerobic conditions. The uptake of this substance is slight, but it is clear that here too aerobic respiration is necessary for the absorption. In experiments with this alkaline-reacting substance we encounter the difficulty that owing to the secretion of acid by the tentacles the pH is altered during the experiment and there will be found di-potassium phosphate by the side of mono-potassium phosphate.

The absorption of phosphate by the DROSERA tentacles reminds us of the absorption of phosphates by sugar-cane, about which a research was made by VAN DEN HONERT. He found that in greatly diluted concentrations the uptake by the roots depends on the phosphate concentration of the liquid nutrient, but that with stronger concentrations than 1 mg per liter the absorption does not increase. Suffice it to point out here the correspondence between the uptake of phosphate by the root and the transport by the tentacle of DROSERA. There are more points of correspondence, which however require a special discussion.

§ 4. *Summary and Discussion.*

In the preceding publication it had been found that the absorption and the transport

of asparagine by the tentacles are processes which only occur in the presence of oxygen. Here it is shown that amino-acids, glycocoll, alanine and leucine behave in the same way and are actively transported like asparagine.

For these substances the not active transport by diffusion is probably but extremely slight, because for these substances the protoplasm is not permeable.

Other organic substances as urea, methyl-urea, thio-urea, phenyl-urea, urotropine, caffeine are in the presence of oxygen transported more or less actively as well. The degree of activation depends on the nature of the substance and is probably greater for substances which cannot diffuse so well through the protoplasm, either on the ground of their dissociation or because of their great molecular volume or owing to a low solubility in lipoids. The activation decreases in the series urea, methyl-urea, thio-urea, phenyl-urea, urotropine, caffeine.

The tentacles also absorb inorganic substances. The uptake of KH_2PO_4 is entirely analogous to that of dissociated organic substances, as amino-acids and asparagine. It is also an accumulation process, which is dependent on oxygen.

The data on the transport mentioned here can be explained, if we assume that the transport consists of two processes. In the first place there is a diffusion process as far as the substances permeate through the protoplasm. These substances are then spread by diffusion in the protoplasm and the vacuole. As the vacuole offers the least resistance and takes up the greater part of the cell volume, the diffusion through the vacuole of well-permeating substances will be the most important process. In addition to diffusion an active transport takes place. The active transport will be more pronounced according as the substances permeate less well through the protoplasm. In the slightly permeating salts, amino-acids and asparagine there is hardly anything but active transport. In those cases in which diffusion and active plasmatic transport take place side by side, the term activated transport has been used. This activated transport was found for urea, methyl-urea, thio-urea, phenyl-urea and caffeine.

For a theoretic discussion of these processes we must refer to a following publication.

Medicine. — *Simplification of the determination of serum albumin and globulin by the spreading method.* By E. GORTER and J. J. HERMANS.

(Communicated at the meeting of September 26, 1942.)

In the determination of serum albumin and globulin by means of spreading as developed by GORTER and BLOKKER [1]), total protein and globulin are measured directly, while the albumin content is calculated from the difference between the spreading areas for both. To spread the globulins, these are precipitated, washed three times in the centrifuge and finally solved in 1 % sodium chloride.

It is obvious that this procedure takes up much time, and we have therefore tried to simplify matters by spreading the albumins directly from the centrifugate, so that the globulins instead of the albumins are determined indirectly. All experiments were carried out with only one tenth the amount of serum mentioned by GORTER and BLOKKER: 0.10 cc of serum are blown from a pipette into 1.0 cc of 1 % NaCl-solution and thoroughly mixed. 5 mm^3 of the mixture are spread on HCl 0.1 N and the area determined. Using the value 1.01 m^2/mg, the protein content is calculated. Further, 0.10 cc of serum are mixed with 0.10 cc of saturated ammoniumsulphate. After centrifuging at 4000 revolutions per min. for about ten minutes, 0.10 cc of the centrifugate are diluted with 1.0 cc of distilled water; 10 mm^3 of this mixture are spread on HCl 0.1 N. With the value 1.04 m^2/mg this leads to the albumin content of the serum.

In this case particular care must be taken to spread as slowly as possible. If the liquid is blown from the pipette too rapidly, it tends to disappear under the surface on account of its high salt content [2]). We want to emphasize this point since its neglect may lead to results which are too low by as much as 20 % or even more. It is quite simple, however, to acquire the technique of spreading sufficiently slowly, and no errors are to be feared once this point is taken into account. It may be said at this juncture that, satisfactorily though the method worked if applied to serum, no reproducible results could be obtained with cerebrospinal liquid. Here the protein content is too low in comparison with the ammonium sulphate present, causing the direct spreading of albumin to break down in almost all cases examined.

The difference between total protein and albumin gives the figure for globulin. If the method is reliable, the direct determination of globulin must tally with that calculated. To show this, the globulins are washed three times with half-saturated ammonium sulphate, finally dissolved in 0.95 cc of 1 % sodium chloride and spread on HCl 0.1 N (10 mm^3 or 20 mm^3 as the case may be). The spreading factor used here is 0.93 m^2/mg. It is assumed that the precipitated globulins with the adhering solution of ammonium sulphate, when dissolved in 0.95 cc of NaCl-solution, make up to very nearly 1 cc, or, in other words, that the final dilution is 10 times. This assumption doubtless is attended with some uncertainty in the results obtained, and it is even difficult accurately to estimate the error involved. Yet it would seem that this error will not be larger than, say, 5 % and that the very small amount of serum needed for the determination amply makes up for this small loss of accuracy.

---

[1]) E. GORTER and P. C. BLOKKER, Proc. Acad. Amsterdam.

[2]) It is clear that we may also dilute the centrifugate with 2 cc of water instead of 1 cc and then spread 20 mm^3 instead of 10. And so on. There is a limit, however, to this procedure. For, if a large quantity of liquid is to be spread slowly, the pipette used must be of small crosssection. In the long and narrow pipettes, however, the surface of the inner wall becomes so large that adsorption of proteins on to this wall begins to play a part. For this reason we have chosen the figure 10. If necessary one may go as far as 20.

All experiments were carried out in duplo. The table gives albumin, globulin and total protein as determined by means of spreading. The fourth column gives the sum of albumin and globulin, showing that the agreement is quite satisfactory. This agreement is the more convincing if we remember that the experimental error involved in the determination of any of the serum proteins mentioned by the spreading method may amount to some 2 %.

Proteins in human serum [3]).

	Albumin %	Globulin %	Total protein %	Total protein calculated (alb. + glob.)
1	4.6	2.10	6.8	6.7
2	4.5	1.92	6.4	6.4
3	4.5	1.60	6.3	6.1
4	4.15	1.30	5.6	5.4
5	4.2	1.75	6.3	6.0
6	6.0	1.65	7.4	7.6
7	5.5	1.70	7.2	7.2
8	5.1	1.60	6.6	6.7
9	4.8	2.0	6.6	6.8
10	5.9	1.65	7.6	7.6
11	5.3	1.74	7.0	7.0
12	4.0	2.1	6.0	6.1

We may conclude that serum albumin may be determined by spreading directly, calculating the globulin content of the serum as the difference between total protein and albumin. In this way the tedious manipulations involved in washing the globulins are avoided, thus reducing the time of an experiment from about 2 hours to, say, three quarters of an hour. There is, of course, one drawback to this method. The globulins represent only $^1/_5$ to $^1/_3$ of the total proteins in serum. An error of 2 % in the determination of albumin may sometimes give rise to an error of 8 % in the globulin calculated. For clinical purposes this is usually of no great consequence. It shows, however, that a direct determination of globulin is to be recommended if the globulin content is wanted with greater precision. Even then, however, our method will be useful since it affords a simple means to check the results obtained.

---

[3]) To avoid confusion, it is to be noted that the results mentioned in this table apply to patients, all children, with a variety of diseases and are not to be considered as average values for normal human serum.

Medicine. — *The spreading of* MACHEBOEUF*'s lipoprotein.* By E. GORTER and J. J. HERMANS.

(Communicated at the meeting of September 26, 1942.)

By repeated precipitation from horse serum MACHEBOEUF [1]) in 1929 succeeded in isolating a water soluble substance containing 59.3 % protein and 40.7 % lipid. After about seven precipitations the composition of this substance remained constant. Since MACHEBOEUF examined the ninth precipitate in particular, he designated this product as A9. The lipids present are lecithin and cholesterol. According to MACHEBOEUF the lecithin content is 22.6 ± 0.3 %. As regards the cholesterol content, it is not quite clear from his paper whether it amounts to 14.0 ± 0.3 % [2]) or 11.2 ± 0.5 % [3]). The lipid could only be separated from the protein by boiling with alcohol, thereby denaturing the protein. For several reasons MACHEBOEUF assumed this protein to be serum albumin, although no definite identification could be achieved.

The procedure followed by us was the one described by MACHEBOEUF. 300 cc of horse serum were mixed with 300 cc of saturated ammonium sulphate. The globulins were filtered off and the filtrate acidified with sulphuric acid to $p_H = 3.8$. The yellow precipitate obtained was filtered off from the colourless filtrate and dissolved in 75 cc of water to which ammonia was added to bring the final p_H up to 7.5 — 8. The clear solution was again acidified with sulphuric acid ($p_H = 3.8$), the precipitate separated in the centrifuge at 4000 revolutions per minute and dissolved at $p_H = 7.5 — 8$. This procedure was repeated eleven times. Usually 10 minutes at 4000 revolutions sufficed to obtain a clear centrifugate, except for the third and fourth precipitate which had to stand over-night in the ice-box before centrifuging. The eleventh precipitate was dissolved in dilute ammonia and dialysed against distilled water at 0° till it was free from ammonium ions.

By evaporating to dryness on a water-bath and finally drying in vacuo above sulphuric acid it was found that this solution contained 5.30 mg dry substance per cc. To determine the lipid content, the solution was boiled with alcohol, the precipitated protein filtered off and washed with alcohol and ether, the filtrate evaporated to dryness and the residue weighed. The lipid content was 2.10 mg per cc, i.e., 39.6 % of the dry substance.

Cholesterol was determined by a colorimetric method, using the reaction of LIEBERMANN–BURCHARD with the anhydride of acetic acid in chloroform. We found 69.4 mg % in the solution, or 13.1 % of the dry substance.

Finally, the nitrogen content was determined colorimetrically using NESSLER's reaction as described by DEMENIER [4]). We found 0.51 mg nitrogen per cc, which is 9.6 % of the dry substance, in good agreement with the nitrogen content calculated for MACHEBOEUF's A9. In fact, this substance should contain 10.0 % nitrogen: 0.6 times 16 for the protein and 0.23 times 1.8 for the lecithin.

The spreading technique was the one developed by GORTER and collaborators [5]).

---

[1]) M. A. MACHEBOEUF, Bull. Soc. Chim. Biol. **11**, 268, 485 (1929); Bull. Soc. Chim. **45**, 663 (1929).

[2]) Reference 1, page 281.

[3]) Reference 1, p. 291 and p. 487.

[4]) G. M. DEMENIER, thesis, Bordeaux 1934.

[5]) E. GORTER and collaborators, Proc. Ned. Akad. v. Wetensch., Amsterdam, **37**, 788 (1934); **29**, 371 (1926).

With p_H below 2.6 the liquid in the tray was dilute HCl. For $p_H > 4$ we used acetate-veronal buffers 0.0033 molar as described by MICHAELIS [6]). With p_H between 2.6 and 3.3 dilute HCl was applied with the addition of NaCl to attain an ionic concentration of 0.0033 gmol/l. All p_H values were measured with the hydrogen electrode.

In fig. 1 some of the pressure-area curves are recorded. It is seen that the nature of these curves is strongly dependent of p_H. With p_H below 3.5 the film shows the

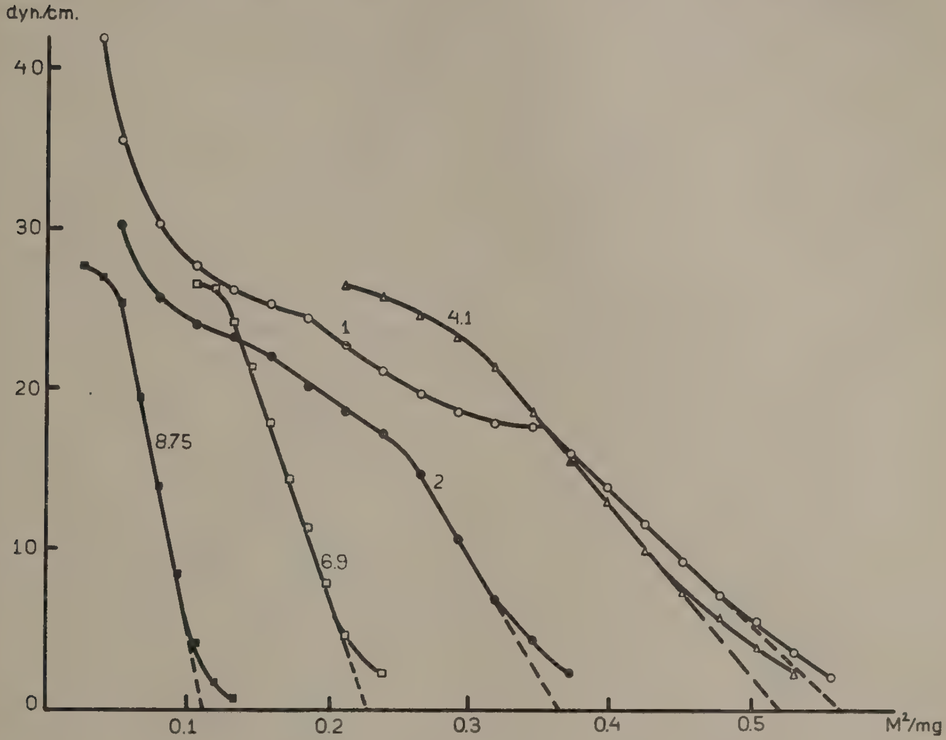


Fig. 1. Pressure-area curves at p_H = 1, 2, 4.1, 6.9 and 8.75.

characteristics of a lipid and may be exposed to high pressures without collapse. If $p_H > 4$, however, the film more and more assumes the characteristics of a protein. This implies that the curvature at pressures beyond about 25 dynes/cm has no definite physical meaning: at these high pressures the film is "folded up", and the pressure drops while compressing. The pressures indicated in this curved part could only be obtained by rapidly compressing the film, and would have been different at a different rate of compression.

The areas obtained when extrapolating the straight part of the curves to zero pressure are plotted against p_H in fig. 2. In this same figure the corresponding areas for serum albumin are recorded, with the only difference that the area is given in square meter per 0.6 mg on account of the fact that MACHEBOEUF's A9 contains about 60 % protein. The striking resemblance between the two curves bears out MACHEBOEUF's assumption that the protein considered is identical with serum albumin.

We further separated the protein from the lipids with the aid of boiling alcohol. Since the protein was denatured in the process, it did spread no longer. The protein was washed with alcohol and ether, the combined filtrates evaporated, and the residue solved

[6]) L. MICHAELIS, Biochem. Z. **234**, 139 (1931).

in petroleum ether. This solution was spread on the tray; its concentration was determined by evaporating the petroleum ether and weighing. For all p_H values the area at zero

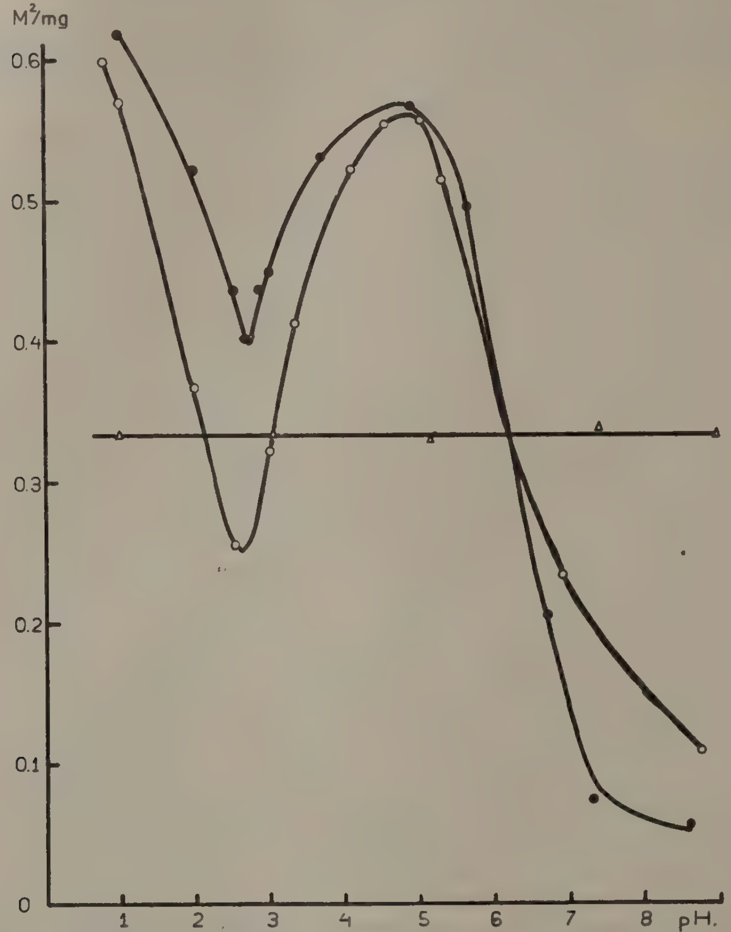


Fig. 2. O MACHEBOEUF's A9
● 60 % serum albumin
△ 40 % lipids

pressure is 0.82 m²/mg. It is interesting to observe that lecithin has a spreading area of 0.86 m²/mg, while that of cholesterol is 0.62 m²/mg. Consequently, a mixture of 2/3 lecithin and 1/3 cholesterol would give 0.78 m²/mg if the spreading values are simply additive. The area found by us thus appears to be quite compatible with MACHEBOEUF's analysis.

In view of the remarkably close resemblance between the area-p_H curves for A9 and serum albumin it would seem that neither the amino groups nor the carboxyl groups of the protein enter into relation with the lipids to any appreciable extent. Yet the existance of a compound is indisputable, since the solution of A9 in water is perfectly clear, whereas, if the lipids after being separated from the protein are shaken with water, a very unstable milky suspension is formed. On the other hand, if the lipids are stirred with a solution of serum albumin in water at $p_H = 8$, they are dissolved, forming a slightly turbid mixture. It was shown, however, by MACHEBOEUF that the lipid is easily

extracted from this mixture by cold ether, whereas the solution of A9 wants a treatment with boiling alcohol for the separation of protein from lipid to be complete. We confirmed this result, and in addition made some spreading experiments with the solution of the lipids in dilute serum albumin, taking care that the protein-lipid ratio was the same as in MACHEBOEUF's A9. It was found that the spreading areas were larger than those of A9. Moreover, the pressure-area curves were quite different. Beyond a certain pressure partial collapse occurred and the protein was squeezed out of the film. A similar behaviour was observed with compounds of oleic acid and ovalbumin which will be described in a later paper.

Medicine. — *The spreading of an ovalbumin-oleic acid complex.* By E. GORTER and J. J. HERMANS.

(Communicated at the meeting of September 26, 1942.)

In view of the results obtained with MACHEBOEUF's lipoprotein from horse serum [1]), it seemed interesting to examine a purely artificial lipoprotein of the type described by PRZYLECKI and HOFER [2]). These authors prepared ovalbumin-oleic acid complexes of varying composition, the largest lipoid content attainable being about 60 %.

10 cc of a 10 % solution of oleic acid in alcohol was added drop by drop from a burette into 120 cc of a 1 % solution of ovalbumin in 0.04 molar borax buffer $p_H = \pm 9$, stirring all the while. The ovalbumin was prepared according to SÖRENSEN [3]). The oleic acid was a KAHLBAUM product, redistilled by us at reduced pressure. The solution obtained was slightly opalescent, its p_H was 8.6. On acidifying with acetic acid to $p_H = 5$, a white precipitate was formed, which was centrifuged off and redissolved in borax. This procedure was repeated six times. For the sake of brevity we will denote the successive precipitates by B_1, B_2 etc. The solutions of B_4, B_5 and B_6 were analysed, and gave the following results for the substance precipitated:

substance	B_4	B_5	B_6
% oleic acid	57.9	60.0	59.2
% protein	42.1	40.0	40.8

The composition of the precipitate obviously is sufficiently constant to treat the substance as a definite compound. The solution of B_6 was spread in the usual way [4]). The areas at zero pressure are given in the figure, where they have been compared with the corresponding ones for ovalbumin. It is seen that the minimum in the area-p_H curve at the acid side has disappeared, showing that the oleic acid combines with the amino groups of the protein. It should further be mentioned that the pressure-area curves below $p_H = 6$ are of the type common to lipids. Collapse of the film does not occur even at pressures which are higher than the collapse point of oleic acid itself. The fact that the ovalbumin thus strengthens the oleic acid film (and vice versa) is obviously of considerable interest to the physical chemistry of natural and artificial membranes.

At $p_H > 7$, however, the films show protein-like behaviour, and pressures larger than about 25 dynes/cm cannot be maintained.

If ovalbumin is made to react with smaller quantities of oleic acid, compounds of varying composition can be obtained [2]). It seemed interesting to study the effect of a larger protein content on the spreading properties of the lipoprotein. To that end only 2 cc of oleic acid, 10 %, in alcohol was slowly added to 60 cc ovalbumin, 1 %, in borax of $p_H = 9$. The resulting solution was acidified with acetic acid, the precipitate redissolved in borax, and so on. For convenience' sake let us denote these precipitates by C_1, C_2, etc. The products C_4 and C_5 were analysed, giving:

substance	C_4	C_5
% oleic acid	40.6	41.3
% protein	59.4	58.7

---

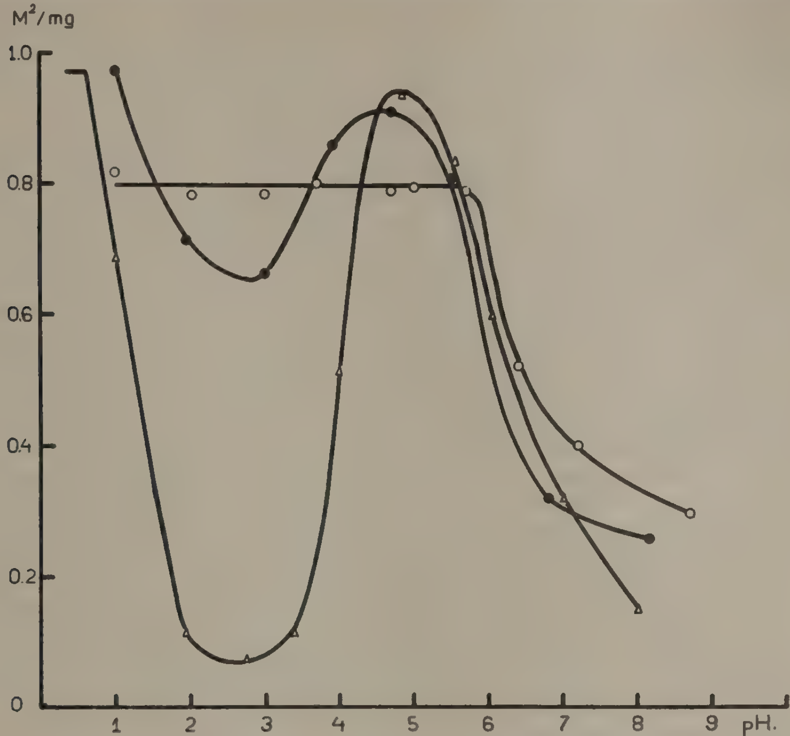
1) E. GORTER and J. J. HERMANS, Proc. Ned. Akad. v. Wetensch., Amsterdam, **45**, 804 (1942).

2) ST. J. V. PRZYLECKI and E. HOFER, Biochem. Z. **288**, 303 (1936); Acta Biol. Exp. **12**, 70 (1938).

3) Compare: E. GORTER, J. V. ORMONDT and F. J. P. DOM, Proc. Kon. Akad. v. Wetensch., Amsterdam, **35**, 838 (1932).

4) E. GORTER and collaborators, Proc. Kon. Akad. v. Wetensch., Amsterdam, **37**, 788 (1934); **29**, 371 (1926).

The spreading areas of C_5 are recorded in the graph. There clearly is a continuous change in the spreading properties of this lipoprotein if the protein content is altered. This



○ B_6, ovalbumin (40 %) — oleic acid (60 %).
● C_5, ovalbumin (59 %) — oleic acid (41 %).
△ ovalbumin.

change is also apparent in the force-area curves. As was to be expected, the force-area curves of C_5 are of the type common to proteins if $p_H > 6$. With p_H below 6, however, the behaviour of C_5 films is quite different from that of B_6. The B_6 films could be compressed to high pressures without collapse. The C_5 films, however, show partial collapse at a certain pressure. Here part of the protein is squeezed out of the monolayer and we are then left with a film which is very similar to the B_6 film. No detailed description of this behaviour will be given here, since pressure-area curves of a similar nature have been studied by SCHULMAN and others [5]) who obtained complex films by injecting substances underneath monolayers.

In the present case it would seem that the protein is only partly squeezed out, since the resulting film is able to resist higher pressures than the oleic acid. This result is similar to that obtained with B_6 and, in fact, the force-area curves of C_5 in this later part are quite compatible with the assumption that no more protein is squeezed out than is needed to bring the lipid content up to about 60 %, the largest figure obtained so far in the ovalbumin-oleic acid compounds prepared by the method described here.

[5]) J. H. SCHULMAN, Proc. Roy. Soc. London, A **155**, 701 (1936).
F. COCKBAIN and J. H. SCHULMAN, Trans. Farady Soc. **35**, 716 (1939).
A. HUGHES, Biochem. J. **29**, 430 (1935).
J. H. SCHULMAN and A. HUGHES, Biochem. J. **29**, 1243 (1935).
J. H. SCHULMAN and E. K. RIDEAL, Proc. Roy. Soc. London, B **122**, 29 (1937); **126**, 356 (1939).

Medicine. — *The spreading of* WARBURG*'s yellow enzyme.* By E. GORTER and J. J. HERMANS.

(Communicated at the meeting of September 26, 1942.)

As an example of a protein with prosthetic group we have spread WARBURG's yellow enzyme prepared from fresh yeast. This substance has been examined by WARBURG and CHRISTIAN [1, 2]), THEORELL [3]), WEYGAND [4, 5]) and others. For convenience sake let us briefly summarize the results obtained by these authors as far as these results are of interest to the present investigation.

The yellow ferment consists of a protein bound to an active group: lactoflavin phosphoric acid. Its molecular weight is $(71 \pm 3) \cdot 10^3$. It contains 15.9 % nitrogen and 0.043 % phosphorus [3]). The protein itself is free from phosphorus. Consequently, since it is very likely that 1 molecule of the active group is combined with 1 molecule of the protein [3]), the molecular concentration of the yellow ferment can be calculated from the molecular phosphorus content.

The yellow ferment owes its colour to the lactoflavin: $C_{17}H_{20}N_4O_6$. As will be described below, we have made use of its absorption of light in the Stufenphotometer, applying the blue filter S 47. We have used KOSCHARA's [6]) value for the extinction: $\varepsilon_1 = 2.80$. This applies to a concentration of 100 γ lactoflavin per cc and a layer of 1 cm. At the time of our experiments we did not know of the value given by KUHN [7]) ($\varepsilon_1 = 3.23$). It was shown, however, by ROTTIER [8]) that KUHN's result must be considered as too high, the actual value being 2.67 ± 0.05. The difference between this latter value and the one used by us is immaterial in view of our imperfect knowledge of the molecular weight and the uncertainties in the determination of nitrogen and phosphorus..

We have prepared the yellow enzyme in the way described by WARBURG and CHRISTIAN [1]).

30 kg fresh yeast from the brewery was washed several times with water. After sedimentation of the yeast, the adhering water was pressed off and the yeast dried at a temperature of 0—5° C. The dry substance was ground till it quantitatively passed a B 20 sieve; we obtained 2 kg of dry powder. This powder was mixed with 7 liter of water, stirred 16 hours at a temperature of about 0° and finally 2 hours at 37°. The yeast was then centrifuged off, and to the liquid obtained (3 l.), 800 cc of lead acetate (liquor plumbi subacetici DAB 6) was added while stirring. After one night at low temperature, the white precipitate was filtered off, the filtrate mixed with 150 cc 1 molar phosphate of p = 7.5, and then filtered again. To the 2.5 l. thus obtained, 1.3 l. of acetone was added at 0°, and the mixture kept at a temperature below zero.

The precipitate was again filtered off and the liquid (3.5 l.) saturated with carbondioxyde and then mixed with 2 l. acetone, always keeping the temperature below zero. An oily precipitate was formed, which was separated from the liquid and then solved in distilled water at 0°.

---

1) O. WARBURG and W. CHRISTIAN, Biochem. Z. **254**, 438 (1932); **266**, 377 (1933).
2) O. WARBURG and W. CHRISTIAN, Biochem. Z. **298**, 367 (1938).
3) H. THEORELL, Biochem. Z. **290**, 293 (1937); **278**, 263 (1935).
4) F. WEYGAND and H. STOCKER, Z. physiol. Chem. **247**, 167 (1937).
5) F. WEYGAND and L. BIRKHOFER, Z. physiol. Chem. **261**, 172 (1939).
6) E. KOSCHARA, Z. physiol. Chem. **232**, 101 (1935).
7) R. KUHN, Ber. deutsch. chem. Ges. **68**, 1765 (1935).
8) P. B. ROTTIER, thesis Delft 1942.

The raw material thus obtained was purified by adsorption and eluation in several stages as described by WEYGAND [4]). It is claimed by WEYGAND and BIRKHOFER [5]) that this process may be directly applied to the aqueous extract of the yeast. Our attempts, however, to obtain the yellow ferment in this way were unsuccessful. We even found it impossible to isolate a pure product if we did not first purify the raw material, prepared in WARBURG's manner, by repeated precipitation with acetone. If this pre-purification was omitted, the process of adsorption and eluation led to a degree of purity of 70 % at most. The adsorption was brought about at $p_H = 5.2$ by a suspension of aluminium-hydroxyde, for the preparation of which we refer to WILLSTÄTTER and KRAUT [9]). Eluation followed in a solution of ammonium sulphate and ammonia at $p_H = 8.2$. The yellow ferment was then precipitated at $p_H = 6$ by ammonium sulphate 50 % at 0° and centrifuged off at 10.000 revolutions per minute, since it was found that a velocity of 4000 revolutions was ineffective. For further particulars the reader is referred to WEYGAND and BIRKHOFER [5]). We may add that both the adsorption and the eluation appeared to be greatly furthered if sufficient time was allowed to elapse in the process (usually 1 or 2 hours).

After 4 adsorptions and eluations the product was practically pure, provided the raw material had first been precipitated 3 times by acetone. The final solution was dialysed against distilled water till it was free from ammonium ions. We obtained 45 cc of a solution containing 4.26 mg pure substance per cc. The concentration was determined and the purity controlled as follows.

a. The nitrogen content of the solution was determined by a micro-Kjeldahl to 0.680 ± 0.003 mg/cc. (Two determinations gave 0.677 and 0.684 respectively). Assuming that no impurities are present, this means that the solutions contained 4.28 ± 0.04 mg yellow enzyme per cc.

b. Phosphorus was determined after a method based on the reaction between phosphate, molybdate and hydroquinone. This method was made applicable to very small quantities of phosphorus by the introduction of the Stufenphotometer.

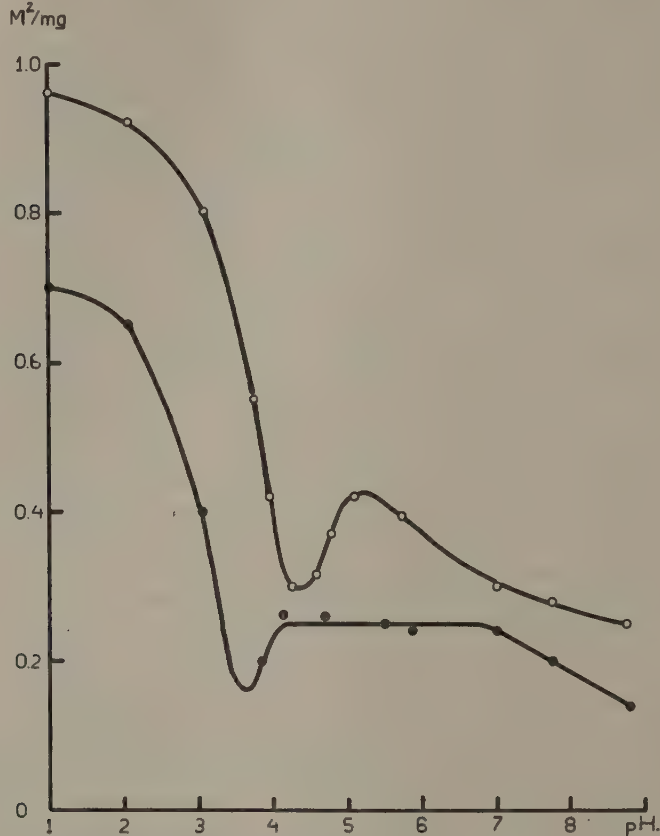
> 4 cc of the solution were destructed by 1 cc concentrated sulphuric acid in a KJELDAHL destruction flask. Hydrogen peroxide 30 % was added to complete the destruction; finally this hydrogen peroxide was removed by the addition of water, and heating. The mixture was then neutralised with NaOH, using phenolphtalein as indicator. After the addition of 2 cc trichloro-acetic-acid 20 %, 0.8 cc ammonium-molybdate 5 % in dilute sulphuric acid and 0.4 cc hydroquinone 0.5 % in dilute $NaHSO_3$, the volume was made up to 10 cc with water, and the mixture kept in the dark for half an hour. The intensity of the blue colour was measured in the Stufenphotometer applying the yellow filter S 57. To find the concentration of phosphate, a reference curve must be made, using different dilutions of a standard phosphate solution. When constructing this curve, care should be taken to add some sodium sulphate to the mixtures, since it appeared that the sodium sulphate formed in the neutralisation of the sulphuric acid to some extent affects the blue colour of the reaction product. We found the method to be quite satisfactory, giving the phosphorus content within a few per cent.

Our final result for the solution of the yellow enzyme was 1.80 ± 0.03 γ phosphorus per cc, i.e., 4.21 ± 0.07 mg yellow ferment per cc. The agreement with the determination of nitrogen is obviously quite satisfactory; we may safely take the concentration to be about 4.26 mg/cc.

c. After diluting the solution ten times, the extinction in the Stufenphotometer with filter S 47 proved to be $\varepsilon_5 = 0.298$ (layer of 5 cm thickness). Or, after reducing to a 1 cm layer: $\varepsilon_1 = 0.0596$. With KOSCHARA's value 2.80 for 100 γ lactoflavin per cc, we

[9]) R. WILLSTÄTTER and H. KRAUT, Ber. deutsch. chem. Ges. **56**, 1117 (1923), **57**, 1082 (1924).

find a concentration of 2.13 γ lactoflavin per cc. Since the molecular weight of lactoflavin is 376, and that of the yellow ferment is $(71 \pm 3) 10^3$, this concentration corresponds to 0.402 ± 0.016 mg yellow ferment per cc. That is, in the original solution, 4.02 ± 0.16 mg/cc. Accordingly, the purity of the product amounts to some 94 %. If we had used ROTTIER's



O Yellow enzyme, isoelectric point 5.22 [3]).
● Protein separated from the yellow enzyme, isoelectric point 5.78 [3]).

value for the extinction, we would have found 4.21 ± 0.17 mg ferment per cc, or 99 %. We have also measured the extinction of the lactoflavin after separation from the protein (see below). This led to the same value for the concentration within experimental error.

The spreading was performed in the usual way [10]). The solution was slowly blown out of a pipette on to the surface. After 1 minute [11]) the film was compressed and the pressure-area curve determined. They were all of the type common to proteins. Extrapolating the straight part of the curve, we obtained the area of the film at zero pressure

---

[10]) E. GORTER and collaborators, Proc. Kon. Akad. v. Wetensch., Amsterdam, **37**, 788 (1934); **29**, 371 (1926).

[11]) In the immediate neighbourhood of the iso-electric point reproducible results could be obtained only if two minutes or more elapsed before compression. Outside the iso-electric region 1 minute suffices; in the iso-electric point, however, spreading appears to be slightly more complete after 2 minutes, without increasing further if this time is increased to 3 minutes or more. For the influence of time on the spreading compare also: ref. 10.

for different p_H-values. These areas are recorded in the graph. Below $p_H = 3.5$ the liquid in the tray was dilute HCl. With p_H between 2.5 and 3.5 sodium chloride $\pm$ 0.002 n was added to attain an ionic concentration comparable to that of the higher p_H-values. For p_H larger than 3.5 we used acetate-veronal buffers 0.0033 molar. All p_H-values were measured by the potentiometric method (hydrogen electrode against calomel electrode) and are accurate to 0.01 units.

The graph further gives the corresponding areas for the protein alone. This protein was obtained in the way described by WARBURG and CHRISTIAN[12]): 10 cc of the solution containing 4.26 mg yellow ferment per cc were mixed with 10 cc saturated ammonium sulphate at 0°. No precipitate was formed. Through the further addition of 4 cc HCl 0.1 n, the protein was separated from the active group, and precipitated by the ammonium sulphate present. After centrifuging at 12.000 revolutions per minute, the protein was washed with half-saturated ammonium sulphate and finally solved in 6 cc 0.03 molar phosphate buffer of $p_H = 7.5$. This solution was dialysed against the said phosphate buffer till it was free from ammonium ions. The nitrogen content was determined by a micro-Kjeldahl and multiplied by a factor 6.25 to give the protein content. The spreading was brought about as usual. Here again it appeared that the spreading time is slightly larger in the iso-electric point (compare note 11). Finally, we may add that the prosthetic group when separated from the protein did not spread at all.

It is seen in the graph that spreading is more complete if the prosthetic group is present in the molecule. It is worth noting that this also applies to p_H-values below 3. In the bulk phase the protein is separated from the prosthetic group if $p_H < 3$. Apparently this reaction does not, or only incompletely, take place in the film, since otherwise no difference should be observed below $p_H = 3$ in the spreading of the protein alone and the protein bound to the active group.

The fact that the prosthetic group itself does not spread is interesting from a biological point of view. One of the functions of the protein in the yellow ferment thus appears to be its ability to spread, thereby greatly furthering the reactivity of the active group in all kinds of biological systems.

It would seem to us that no very detailed conclusions can as yet be drawn regarding the nature of the bonds between protein and prosthetic group, as could be done in the case of several other complex proteins[12]). From the fact that the minimum on the acid side is shifted to larger p_H-values if the prosthetic group is present, it might be inferred that the prosthetic group enters into relation with some of the NH_2-groups or other basic groups of the protein. The depth of this minimum, however, is not altered to a very pronounced extent and some caution is therefore desirable as regards the precise meaning of this shift to larger p_H.

[12]) E. GORTER and L. MAASKANT, Proc. Kon. Akad. v. Wetensch., Amsterdam, **40**, 71 (1937); E. GORTER, Trans. Faraday Soc. **33**, 1125 (1937); E. GORTER, H. v. ORMONDT and TH. M. MEYER, Biochem. J. **29**, 38 (1935).

Physics. — *On the influence of the condensor on the resolving power of a microscope.* By T. Y. KINGMA BOLTJES and C. J. GORTER. (Laboratorium voor Microbiologie; ZEEMAN-Laboratorium der Universiteit van Amsterdam.) (Communicated by Prof. P. ZEEMAN.)

(Communicated at the meeting of September 26, 1942.)

§ 1. *Introduction.* ABBE's famous treatment leads to the following expression for the resolving power $\triangle$ of a microscope used with parallel incident light

$$\triangle = \lambda / A, \quad \ldots \ldots \ldots \quad (1)$$

where λ is the wavelength in vacuo of the light and A the numerical aperture of the objective lens system. In his derivation of this expression ABBE supposes the object to be a transmission-grating with a large number of elements and a period d. This grating is supposed to be perpendicular to the optical axis of the instrument and if now the incident light beam is parallel to the optical axis, the diffracted beams make angles ϑ with this axis, where $\mu\, d \sin \vartheta = \pm\, n \lambda$, being μ the refractive index and n a positive whole number indicating the order of the diffracted beam. The condition that, in addition to the central beam ($n = 0$), both diffracted beams of the first order ($n = 1$) pass through the entrance pupil of the objective, so that they can contribute to the formation of the image, gives: $\sin \vartheta_1 = \lambda / \mu\, d \leq A / \mu$ and so leads to $d \geq \lambda / A$. Thus in fact $\triangle = \lambda / A$ is the minimum period of the grating that can be resolved.

If oblique incident light is used it is possible that the central beam and one of the two first order beams pass through the objective. This will also bring about resolution of the structure (see § 2). In the most favorable case the incident beam makes an angle $\arcsin A/\mu$ with the optical axis and lies in the plane perpendicular to the lines of the grating. Then the condition is: $\sin \vartheta_1 = \lambda / \mu d - A/\mu \leq A/\mu$ and so $d \geq \lambda / 2A$. Therefore, if the aperture of the condensor is taken at least as large as that of the objective, the expected resolving power is $\lambda / 2A$. Though in practice (see § 3) the use of a condensor certainly gives a considerable gain in resolving power, the gain is less than the factor 2 given in several textbooks. It has been stressed by VAN CITTERT[1]) that this is due to the fact that the eye is not able to resolve a structure if the visibility is too low. The visibility V is defined by

$$V = \frac{I_{ma} - I_{mi}}{I_{ma} + I_{mi}}, \quad \ldots \ldots \ldots \quad (2)$$

where I_{ma} and I_{mi} indicate the intensities at the light maxima and minima in the image respectively.

The point of view of the present note is somewhat different from VAN CITTERT's reasoning. In an elementary theoretical treatment we will try to connect the visibility of the image with the FOURIER-components characterizing the object and we will compare the results with observations on different objects. In this connection we will also discuss the effect of a central screen in the condensor system giving rise to all-sided oblique illumination.

§ 2. *Elementary theory.*

Let us suppose an incident beam of light characterized by $B_0 \cos 2\pi\nu(t - \mu z/c)$ in the direction of the optical axis (z-direction). The grating is orientated in the (x, y) plane,

---

1) P. H. VAN CITTERT, Proc. Kon. Akad. v. Wetensch., Amsterdam, **39**, 182 (1936).

its lines being parallel to the y direction, its transmission T for the lightvector being

$$T = \sum_n D_n \cos\left(\frac{2\pi n x}{d} - \varphi_n\right), \quad (3)$$

where the summation extends over the positive whole numbers, 0 included.

The lightvector $B(\vartheta)$ diffracted into a direction lying in the (y, z) plane and making an angle ϑ with the z-direction is:

$$\left.\begin{aligned} B(\vartheta) = B_0 \int_0^l \sum_n D_n \cos\left(\frac{2\pi n x}{d} - \varphi_n\right) \cos\left(2\pi\nu t - \frac{2\pi \mu x \sin\vartheta}{\lambda}\right) dx = \\ B_0 \int_0^l \sum_n \frac{D_n}{2} \cos\left\{2\pi\nu t - 2\pi x\left(\frac{\mu \sin\vartheta}{\lambda} - \frac{n}{d}\right) - \varphi_n\right\} dx + \\ + \sum_n \frac{D_n}{2} \cos\left\{2\pi\nu t - 2\pi x\left(\frac{\mu \sin\vartheta}{\lambda} + \frac{n}{d}\right) + \varphi_n\right\} dx, \end{aligned}\right\} \quad (4)$$

where l, being a multiple of d, is the length in the x-direction of the grating.

The integration in (4) gives a value different from zero only if

$$\frac{\mu \sin\vartheta}{\lambda} \pm \frac{n}{d} = 0. \quad (5)$$

For these values of ϑ we get

$$B(\vartheta) = \frac{B_0 D_n l}{2} \cos(2\pi\nu t \pm \varphi_n) \quad (6)$$

being one of the two diffracted beams of the n^{th} order.

Let us now first take the case considered in the very beginning of this note that the central beam $(n = 0)$ and both first order beams $(n = 1)$ are passing through the objective. Then apart from an unessential geometrical factor and an also unessential phase difference we get for the lightvector in the image:

$$\left.\begin{aligned} B_i = D_0 \cos 2\pi\nu t + \frac{D_1}{2} \cos\left(2\pi\nu t - \varphi_1 + \frac{2\pi x'}{d'}\right) + \\ + \frac{D_1}{2} \cos\left(2\pi\nu t + \varphi_1 - \frac{2\pi x'}{d'}\right), \end{aligned}\right\} \quad (7)$$

where x' and d' indicate the image of x and d.

The intensity I_i being the mean square of B_i, is

$$I_i = \frac{1}{2}\left[D_0 + D_1 \cos\left(\frac{2\pi x'}{d'} - \varphi_1\right)\right]^2 \quad (8)$$

and the visibility of the image

$$V = \frac{I_{ma} - I_{mi}}{I_{ma} + I_{mi}} = \frac{2 D_0 D_1}{D_0^2 + D_1^2}. \quad (9)$$

If however the central beam and only one of the first order beams pass we have to

preserve only the two first terms in the right hand side of (7) and we find, as may be verified easily:

$$I_i = \frac{1}{2}\left[D_0^2 + \frac{D_1^2}{4} + D_0\,D_1 \cos\left(\frac{2\pi x'}{d'} - \varphi_1\right)\right] \quad . \;\; . \;\; . \quad (10)$$

and

$$V = \frac{4\,D_0\,D_1}{4\,D_0^2 + D_1^2}. \quad . \;\; . \;\; . \;\; . \;\; . \;\; . \;\; . \;\; . \;\; . \;\; . \quad (11)$$

If for example $D_1 = D_0$ which means that, as far as these two FOURIER-coefficients are concerned, the visibility of the object is 1, the visibility of the image according to (9) is of course 1 too, whilst it is 0.8 according to (11) if the central beam and only one of the first order beams are effective.

In the elementary treatment just given it was not at all essential that the incident light was parallel to the z-direction; if the incident light has a different direction only the expressions for the directions of the diffraction beams are less simple than (5) but the formulae (6) to (11) remain valid[1]).

Let us now suppose the incident light to consist of a number of independant beams coming from different directions such as approximately is realised by the use of a condensing system of wide aperture. And let us suppose that from a fraction α of those beams the central order and one of the first order diffraction beams pass through the objective whilst from the remaining fraction $1 - \alpha$ merely the central order passes.

Then the intensity observed (apart from unessential numerical factors) will be:

$$I_i = \alpha\left[D_0^2 + \frac{D_1^2}{4} + D_0\,D_1 \cos\left(\frac{2\pi x'}{d'} - \varphi_1\right)\right] + (1-\alpha)\,D_0^2 \quad . \quad (12)$$

and correspondingly

$$V = \frac{4\,\alpha\,D_0\,D_1}{4\,D_0^2 + \alpha\,D_1^2}. \quad . \;\; . \;\; . \;\; . \;\; . \;\; . \;\; . \;\; . \;\; . \;\; . \quad (13)$$

So the visibility in this case depends on α and on D_1/D_0; if $\alpha D_1^2 \ll 4D_0^2$ the visibility will be proportional to both these numbers. In this D_1/D_0 only characterizes the contrast of the grating structure and α depends on the arrangement and apertures of the condensor and the objective and of the period d of the grating.

In order to get an idea of the value of α let us consider fig. 1 where c indicates the aperture circle of the condensor and the dotted circle o indicates the aperture circle of the objective which is supposed to be at least as large. Every point inside the circle o indicates an incident beam, the intensity of a group of beams being proportional to the corresponding area in the diagram. The grating has its lines vertical in the plane of the paper. One of the first orders arising from some of the incident beams just passes through the circumference of the entrance pupil of the objective. The lines l are the locus of those incident beams. If the apertures are small, the lines may be found by displacing the objective circle (representing the entrance pupil in question) by a distance λ/d to the right and to the left. Calculation shows that in the case of large apertures the lines l are less curved than the corresponding parts of the circle o, but the error made by taking circles instead of the correct l-lines usually is quite small.

Now all points inside the circle c correspond to an incident beam and so α is the ratio between the area of the hatched part of the circle and the whole circle. If $d = \lambda/2A$ the l-curves touch the c-circle externally and $\alpha = 0$. If then d increaces, α grows until

---

[1]) Of course the FOURIER-coefficients D may be somewhat dependant on the direction. This complication has been neglected in the present treatment.

for $d = \lambda/A$ the l-curves touch each other in the centre. For still larger values of d some incident beams give rise to two first orders passing through the objective and the present reasoning is not valid.

When d lies between $\lambda/2A$ and λ/A none of the first orders arising from an incident beam near the centre passes through the objective. Therefore in general it is of advantage to block those beams by placing a central screen in the condensor aperture, thus causing allsided oblique illumination. From fig. 2 in comparison to fig. 1 it is seen that in this

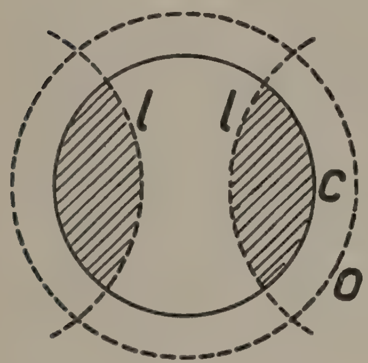


Fig. 1.

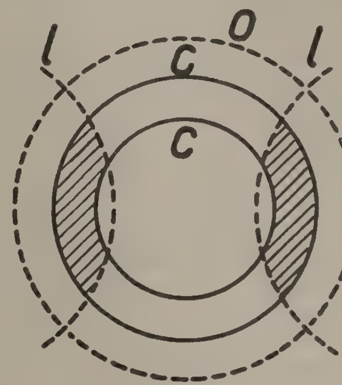


Fig. 2.

way the value of α is considerably increased. The advantage of such an illumination for special purposes has been recognized by microscopists at an early date and has already been explained by STONEY and SIEDENTOPF[1]). In this respect one must not forget that the introduction of a central screen in the condensor is a plain disadvantage in so far as the object contains structures wider than λ/A.

§ 3. In order to test the elementary theory given in § 2 we performed a few series of experiments in which we used a GRAYSON's ruling, kindly placed at our disposal by Dr. P. H. VAN CITTERT, and various diatoms as grating-objects.

In these experiments we made use of a Zeiss microscope fitted out with an aplanatic condensor ($A_{max} = 1.4$) and an achromatic Zeiss objective ($A_{max} = 1.25$) with a small iris diaphragm allowing variation of the aperture of the objective. We calibrated this diaphragm with the aid of an ABBE apertometer, kindly lend to us by Prof. CLAY. The condensor contained a variable diaphragm upon which we could place celluloid discs in the centre of which a painted black circle serves as central screen. The apertures of the condensor and of the central screens could be determined with the calibrated diaphragm in the objective. A $20\times$ compensating ocular was used.

The light source was a Philips Philora lamp 300 H.P. According to KÖHLER's principle the light was concentrated on the condensor with the aid of an aplanatic collector in such a way that the diaphragm of the collector was projected into the field of view but that, in order to diminish the stray light, merely this field of view was illuminated. With the aid of suitable liquid light filters monochromatic light could be obtained.

In the experiments a certain aperture of the condensor and of the central screen were chosen and then the minimum aperture of the objective was determined at which the structure was seen to be resolved. As an example these minimum apertures have been given from series of measurements on the narrowest GRAYSON's ruling with a grating period of $0.436\,\mu$ and a wavelength of $0.546\,\mu$ and on the specks of the diatiom Surirella

---

[1]) G. J. STONEY, J. Science, **42**, 499 (1896).
H. SIEDENTOPF, Z.wiss. Mikroskopie, **33**, 1 (1915).

gemma with a grating period of 0.380 μ and a wavelength of 0.577 μ respectively in the tables I and II.

TABLE I.

Minimum apertures necessary to resolve a GRAYSON's ruling with a grating period of 0.436 μ at a wavelength of 0.546 μ.

A-disc	0	0.1	0.2	0.3	0.4	0.5	0.6
A-condensor							
0.4	0.95	0.94	0.92	0.90			
0.5	0.90	0.89	0.87	0.85	0.83		
0.6	0.82	0.81	0.80	0.78	0.76	0.74	
0.7	0.77	0.76	0.75	0.74	0.72	0.72	0.72

TABLE II.

Minimum apertures necessary to resolve a structure in Surirella gemma with a grating period of 0.380 μ at a wavelength of 0.577 μ.

A-disc	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
A-condensor								
0.4			1.25	1.20				
0.5	1.22	1.22	1.20	1.17	1.16			
0.6	1.16	1.16	1.14	1.12	1.09	1.06		
0.7	1.12	1.12	1.10	1.08	1.06	1.03	0.98	
0.8	1.10	1.10	1.08	1.04	1.02	1.00	0.98	0.98

By making diagrams like fig. 1 and fig. 2 the value of α for every combination of the apertures of objective, condensor and central screen may easily be obtained. In the tables III and IV we give those values of α obtained from the data of tables I and II.

TABLE III.

Values of the α's in the cases of Table I.

A-disc	0	0.1	0.2	0.3	0.4	0.5	0.6
A-condensor							
0.4	0.12	0.11	0.10	0.11			
0.5	0.15	0.14	0.13	0.14	0.17		
0.6	0.13	0.13	0.12	0.12	0.13	0.18	
0.7	0.15	0.14	0.14	0.16	0.18	0.20	0.28

TABLE IV.

Values of the α's in the cases of Table II.

A-disc	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7
A-condensor								
0.4			0.24	0.24				
0.5	0.24	0.25	0.24	0.24	0.27			
0.6	0.24	0.25	0.24	0.24	0.24	0.27		
0.7	0.24	0.25	0.24	0.24	0.25	0.28	0.27	
0.8	0.24	0.24	0.25	0.24	0.24	0.28	0.32	0.33

According to the theory of § 2 the visibility V is connected to the values of α and D_1/D_0 by equation (13). As the observations on the minimum aperture of the objective have all been carried out by one person, (T.Y.K.B.) the minimum visibility V_{min} was approximately the same for all the observations of one series. If one may assume that D_1/D_0 which describes the contrast of the structure under consideration, is constant in one series, equation (13) leads us to expect the same value of α for all the observations in that series. From the tables III and IV it may by seen that this expectation is remarkably well fulfilled as long as the apertures of the central screens are not too large. The deviations at large apertures of condensor and screen mean a lower visibility than expected and may either be due to increased aberrations of the microscope or to the decreased value of D_1/D_0 when we have to do with very oblique light rays [1]) or possibly to both causes [2]). It is hardly necessary to stress that, as the minimum visibility V_{min} which an observer can detect depends on the observer's eye and as the value of D_1/D_0 depends on the wavelength as well as on the nature of the object, the α-values obtained in the tables III and IV have no absolute significance.

The increase in resolving power which in practice may be obtained by the use of the condensor without central screen depends — as has already been pointed out by VAN CITTERT — on the minimum visibility V_{min} which the observer's eye can detect as well as on the contrast of the structure (D_1/D_0). From the data of the tables I and II it may be gathered that the increase in resolving power obtained is a factor 1.6 in the case of the GRAYSON's ruling and a factor 1.4 in the case of Surirella gemma. Experiments with still finer structures showed that even at very high apertures of the objective this factor remains of the same order. In the diatom Amphipleura pellucida mounted in realgar (this gives a very good contrast) a structure with a period of 0.260 μ could be resolved with A-condensor $= 1.1$ and A-objective $= 1.3$ at a wavelength of 0.577 μ. The gain due to the condensor then is a factor 1.7. Though these factors are all considerably lower than 2, they are much higher than those obtained by VAN CITTERT (about 1.2). This difference probably will partly be due to the better illumination we used. The fact that we used a condensor which was aplanatic proved to be of rather little importance for this factor [3]).

Zusammenfassung: Es wird in Anschluss an die ABBEsche Abbildungslehre eine elementare Theorie gegeben für die Sichtbarkeit der Abbildung von periodischen Strukturen. Diese Theorie wird angewandt auf den Einfluss des Kondensors im Mikroskop auf die Sichtbarkeit, wobei auch die Anwendung einer zentralen Blende im Kondensor besprochen wird. Durch Beobachtungen an einem GRAYSON–GITTER und einigen Diatomeen werden die theoretischen Resultate bestätigt. Bei richtiger Beleuchtung zeigt sich der Vorteil des Kondensors beträchtlich kleiner als viele Lehrbücher angeben, aber grösser als früher von VAN CITTERT gefunden wurde.

---

[1]) See footnote on page 816.

[2]) Note added in the proof: Probably this phenomenon is also partly due to vanishing phase contrast. Comp. F. ZERNIKE, Physica 9, 696 (1942).

[3]) The difference between an aplanatic and an uncorrected condensor immediately becomes apparent when a black circle is laid upon the diaphragm of the condensor. The aplanatic condensor gives a very good dark field illumination even with a strong objective whereas the dark field of the uncorrected condensor is very poor.

Mathematical Physics. — *Sur l'intégration de quelques problèmes aux limites régis par l'équation de* FOURIER *dite „de la chaleur" au moyen de la méthode des transformations fonctionnelles simultanées.* Par S. R. DE GROOT. (Quatre-vingtième publication du „Fonds VAN DER WAALS", VAN DER WAALS Laboratorium, Gemeente-Universiteit, Amsterdam.) (Communicated by Prof. J. D. VAN DER WAALS JR.)

(Communicated at the meeting of September 26, 1942.)

Sommaire. La méthode des transformations fonctionnelles simultanées est très bien adaptée à l'intégration de problèmes aux limites sur la propagation de la chaleur dans un fil. Le choix des transformations à effectuer est déterminé par les intervalles des variables et par les conditions aux limites. Pour résoudre des problèmes à conditions aux limites de 2ième ou 3ième espèce on doit parfois faire appel à des transformations de FOURIER peu usitées, ainsi qu'à une transformation de LAPLACE.

II. Condition aux limites de deuxième et troisième espèce.

Dans une note précédente (1) nous avons montré par quelques exemples empruntés à la théorie de la chaleur, que le calcul des transformations fonctionnelles simultanées fournit une méthode rapide et simple pour trouver la solution de problèmes avec des conditions aux limites de première espèce. Le présent travail est consacré à l'étude de la même méthode appliquée à quelques problèmes avec des conditions aux limites d'un caractère différent.

§ 6. *Le conducteur fini avec température initiale donnée, tandis qu'aux extrémités la radiation est donnée (conditions aux limites de deuxième espèce).*

Le problème qui se pose ici, est le suivant:

$$\frac{\partial^2 U}{\partial x^2} = \frac{\partial U}{\partial t}, \quad \ldots \ldots \ldots \ldots \quad (45)$$

$$\lim_{t \to 0} U(x, t) = K(x), \quad \ldots \ldots \ldots \quad (46)$$

$$\lim_{x \to 0} \frac{\partial U}{\partial x} = B_0(t), \quad \ldots \ldots \ldots \quad (47)$$

$$\lim_{x \to 1} \frac{\partial U}{\partial x} = B_1(t). \quad \ldots \ldots \ldots \quad (48)$$

Employons la transformation de LAPLACE par rapport au temps, puisque l'intervalle de la variable t est $(0, \infty)$. Pour l'autre, celle par rapport à x avec l'intervalle $(0,1)$, nous prenons a transformation de FOURIER:

$$\underset{x}{\mathfrak{F}}\, U(x, t) = 2 \int_0^1 U(x, t) \cos n\pi x \, dx, \quad \ldots \ldots \quad (49)$$

qui est l'inverse de la série des cosinus de FOURIER:

$$U(x, t) = \tfrac{1}{2} [\underset{x}{\mathfrak{F}}\, U(x, t)]_{n=0} + \sum_{n=1}^{\infty} [\underset{x}{\mathfrak{F}}\, U(x, t)] \cos n\pi x. \quad \ldots \quad (50)$$

La raison, pour laquelle nous choisissons cette transformation est que le théorème de différentiation en est pour la dérivée du second ordre:

$$\underset{x}{\mathfrak{F}}\frac{\partial^2 U}{\partial x^2}=-n^2\pi^2\underset{x}{\mathfrak{F}}U(x,t)-2B_0(t)+2(-1)^n B_1(t). \quad (51)$$

Le second membre contient les dérivées de U aux extrémités et non pas les valeurs de U lui-même. C'est là justement ce que les conditions aux limites demandent. L'équation transformée devient:

$$-n^2\pi^2\underset{t}{\mathfrak{L}}\underset{x}{\mathfrak{F}}U(x,t)-2\underset{t}{\mathfrak{L}}B_0(t)+2(-1)^n\underset{t}{\mathfrak{L}}B_1(t)=p\underset{t}{\mathfrak{L}}\underset{x}{\mathfrak{F}}U(x,t)-\underset{x}{\mathfrak{F}}K(x) \quad (52)$$

et sa solution:

$$\underset{t}{\mathfrak{L}}\underset{x}{\mathfrak{F}}U(x,t)=\frac{-2\underset{t}{\mathfrak{L}}B_0(t)+2(-1)^n\underset{t}{\mathfrak{L}}B_1(t)+\underset{x}{\mathfrak{F}}K(x)}{p+n^2\pi^2}. \quad (53)$$

En appliquant la transformation inverse par rapport à t, on trouve:

$$\underset{x}{\mathfrak{F}}U(x,t)=2e^{-n^2\pi^2 t}*[-B_0(t)+(-1)^n B_1(t)]+e^{-n^2\pi^2 t}\underset{x}{\mathfrak{F}}K(x) \quad (54)$$

$$\underset{x}{\mathfrak{F}}U(x,t)=2e^{-n^2\pi^2 t}*[-B_0(t)+(-1)^n B_1(t)]+2e^{-n^2\pi^2 t}\int_0^1 K(\xi)\cos n\pi\xi\, d\xi \quad (55)$$

et de même pour x:

$$\left.\begin{aligned}U(x,t)&=1*[-B_0(t)+B_1(t)]+\\&+2\sum_{n=1}^{\infty}e^{-n^2\pi^2 t}*[-B_0(t)+(-1)^n B_1(t)]\cos n\pi x+\int_0^1 K(\xi)\,d\xi+\\&\qquad+2\sum_{n=1}^{\infty}e^{-n^2\pi^2 t}\int_0^1 K(\xi)\cos n\pi\xi\cos n\pi x\, d\xi,\end{aligned}\right\} \quad (56)$$

d'où, en utilisant les fonctions-thêta:

$$\left.\begin{aligned}U(x,t)&=-\vartheta_3(\tfrac{1}{2}x,t)*B_0(t)+\\&+\vartheta_3(\tfrac{1}{2}-\tfrac{1}{2}x,t)*B_1(t)+\tfrac{1}{2}\int_0^1\left[\vartheta_3\left(\frac{x-\xi}{2},t\right)+\vartheta_3\left(\frac{x+\xi}{2},t\right)\right]K(\xi)\,d\xi.\end{aligned}\right\} \quad (57)$$

Donc la solution contient les fonctions ϑ_3 et non pas les dérivées de ces fonctions, comme dans le § 3. On pouvait s'attendre à ce résultat puisque les derniers termes du second membre du théorème de différentiation (51) ne contiennent pas un facteur n, comme au § 3.

§ 7. *Le conducteur fini, où la température initiale et celle à l'extrémité $x=0$ sont données, tandis qu'à l'autre extrémité la radiation est donnée (un cas spécial de conditions aux limites de troisième espèce).*

Ce problème se formule:

$$\frac{\partial^2 U}{\partial x^2} = \frac{\partial U}{\partial t}, \quad \ldots \ldots \ldots \ldots \quad (58)$$

$$\lim_{t \to 0} U(x, t) = K(x), \quad \ldots \ldots \ldots \quad (59)$$

$$\lim_{x \to 0} U(x, t) = A_0(t), \quad \ldots \ldots \ldots \quad (60)$$

$$\lim_{x \to 1} \frac{\partial U}{\partial x} = B_1(t). \quad \ldots \ldots \ldots \ldots \quad (61)$$

Ici encore pour la variable du temps une transformation de LAPLACE est indiquée. Pour la transformation par rapport à x, dont l'intervalle est $(0,1)$, une transformation de FOURIER s'impose. Mais, comme les conditions aux limites ont des caractères différents, il faut se servir d'une transformation de FOURIER, dont le théorème de différentiation tient compte des conditions aux limites. C'est le cas avec la transformation:

$$\underset{x}{\mathfrak{F}}\, U(x, t) = 2 \int_0^1 U(x, t) \sin\left(n + \tfrac{1}{2}\right) \pi x \, dx, \quad \ldots \quad (62)$$

qui est l'inverse d'une série de sinus avec $(n + \frac{1}{2})$ au lieu de n:

$$U(x, t) = \sum_{n=0}^{\infty} \left[\underset{x}{\mathfrak{F}}\, U(x, t)\right] \sin\left(n + \tfrac{1}{2}\right) \pi x. \quad \ldots \ldots \quad (63)$$

En effet, le théorème de différentiation a la forme:

$$\underset{x}{\mathfrak{F}} \frac{\partial^2 U}{\partial x^2} = -\left(n + \tfrac{1}{2}\right)^2 \pi^2 \underset{x}{\mathfrak{F}}\, U(x, t) + 2\left(n + \tfrac{1}{2}\right) \pi A_0(t) + 2(-1)^n B_1(t) \quad (64)$$

et il contient les grandeurs qui ont été imposées au problème. On trouve de la manière habituelle en effectuant les transformations simultanées:

$$\left.\begin{aligned} -\left(n + \tfrac{1}{2}\right)^2 \pi^2 \underset{t}{\mathfrak{L}} \underset{x}{\mathfrak{F}}\, U(x, t) + 2\left(n + \tfrac{1}{2}\right) \pi \underset{t}{\mathfrak{L}}\, A_0(t) + \\ + 2(-1)^n \underset{t}{\mathfrak{L}}\, B_1(t) = p \underset{t}{\mathfrak{L}} \underset{x}{\mathfrak{F}}\, U(x, t) - \underset{x}{\mathfrak{F}}\, K(x) \end{aligned}\right\} \quad (65)$$

et la solution transformée:

$$\underset{t}{\mathfrak{L}} \underset{x}{\mathfrak{F}}\, U(x, t) = \frac{2\left(n + \tfrac{1}{2}\right) \pi \underset{t}{\mathfrak{L}}\, A_0(t) + 2(-1)^n \underset{t}{\mathfrak{L}}\, B_1(t) + \underset{x}{\mathfrak{F}}\, K(x)}{p + \left(n + \tfrac{1}{2}\right)^2 \pi^2}. \quad (66)$$

Cela étant, on obtient en effectuant les transformations inverses:

$$\underset{x}{\mathfrak{F}}\, U(x, t) = 2 e^{-(n+\frac{1}{2})^2 \pi^2 t} * \left[\left(n + \tfrac{1}{2}\right) \pi A_0(t) + (-1)^n B_1(t)\right] + e^{-(n+\frac{1}{2})^2 \pi^2 t} \underset{x}{\mathfrak{F}}\, K(x). \quad (67)$$

$$\left.\begin{aligned}\underset{x}{\mathfrak{F}}\, U(x,t) &= 2\, e^{-(n+\frac{1}{2})^2\pi^2 t} * [(n+\tfrac{1}{2})\pi A_0(t) + (-1)^n B_1(t)] + \\ &\quad + 2\, e^{-(n+\frac{1}{2})^2\pi^2 t}\int_0^1 K(\xi)\sin(n+\tfrac{1}{2})\pi\xi\, d\xi,\end{aligned}\right\} \quad (68)$$

$$\left.\begin{aligned}U(x,t) &= 2\sum_{n=0}^{\infty} e^{-(n+\frac{1}{2})^2\pi^2 t} * [(n+\tfrac{1}{2})\pi A_0(t) + (-1)^n B_1(t)]\sin(n+\tfrac{1}{2})\pi x + \\ &\quad + 2\sum_{n=0}^{\infty} e^{-(n+\frac{1}{2})^2\pi^2 t}\int_0^1 K(\xi)\sin(n+\tfrac{1}{2})\pi\xi\,\sin(n+\tfrac{1}{2})\pi x\, d\xi,\end{aligned}\right\} \quad (69)$$

ce qui permet d'écrire avec les fonctions ϑ_2:

$$\left.\begin{aligned}U(x,t) &= -\frac{\partial\vartheta_2(\frac{1}{2}x,t)}{\partial x} * A_0(t) + \\ &+\vartheta_2(\tfrac{1}{2}-\tfrac{1}{2}x,t) * B_1(t) + \tfrac{1}{2}\int_0^1\left[\vartheta_2\left(\frac{x-\xi}{2},t\right) - \vartheta_2\left(\frac{x+\xi}{2},t\right)\right] K(\xi)\, d\xi.\end{aligned}\right\} \quad (70)$$

Le fait que le calcul aboutit à des fonctions ϑ_2 pouvait être prévu, puisque la définition de la fonction ϑ_2 contient les mêmes facteurs $(n+\frac{1}{2})$ que notre transformation. Aussi trouve-t-on la dérivée de ϑ_2 dans le terme, qui correspond à l'extrémité où la fonction U est donnée, tandis que pour l'autre extrémité, où la dérivée de U est donnée, une fonction ϑ_2 est trouvée. Ces résultats découlent du caractère du théorème de différentiation (64).

§ 8. *Le conducteur fini, où la température initiale et celle à l'extrémité $x = 1$ sont données tandis qu'à l'autre extrémité $x = 0$ la radiation est donnée (un cas spécial de conditions aux limites de troisième espèce).*

Ce problème est presque le même que le précédent:

$$\frac{\partial^2 U}{\partial x^2} = \frac{\partial U}{\partial t}, \quad \ldots\ldots\ldots\ldots \quad (71)$$

$$\lim_{t\to 0} U(x,t) = K(x), \quad \ldots\ldots\ldots\ldots \quad (72)$$

$$\lim_{x\to 0}\frac{\partial U}{\partial x} = B_0(t), \quad \ldots\ldots\ldots\ldots \quad (73)$$

$$\lim_{x\to 1} U(x,t) = A_1(t), \quad \ldots\ldots\ldots\ldots \quad (74)$$

mais comme les conditions aux limites ont été interchangées, il faut utiliser une autre transformation de FOURIER, à savoir:

$$\underset{x}{\mathfrak{F}}\, U(x,t) = 2\int_0^1 U(x,t)\cos(n+\tfrac{1}{2})\pi x\, dx, \quad \ldots \quad (75)$$

provenant de la série des cosinus avec $(n+\frac{1}{2})$:

$$U(x, t) = \sum_{n=0}^{\infty} [\underset{x}{\mathfrak{F}} U(x, t)] \cos (n+\tfrac{1}{2}) \pi x. \quad \ldots \quad (76)$$

Le théorème de différentiation de cette transformation est:

$$\underset{x}{\mathfrak{F}} \frac{\partial^2 U}{\partial x^2} = -(n+\tfrac{1}{2})^2 \pi^2 \underset{x}{\mathfrak{F}} U(x, t) - 2 B_0(t) + 2(-1)^n (n+\tfrac{1}{2}) \pi A_1(t). \quad (77)$$

Cette propriété permet de résoudre facilement le problème:

$$\underset{t}{\mathfrak{L}} \underset{x}{\mathfrak{F}} U(x, t) = \frac{-2 \underset{t}{\mathfrak{L}} B_0(t) + 2(-1)^n (n+\frac{1}{2}) \pi \underset{t}{\mathfrak{L}} A_1(t) + \underset{x}{\mathfrak{F}} K(x)}{p + (n+\frac{1}{2})^2 \pi^2}. \quad (78)$$

Il est évident que la solution devient:

$$\left. \begin{aligned} U(x,t) = 2 \sum_{n=0}^{\infty} e^{-(n+\frac{1}{2})^2 \pi^2 t} * [-B_0(t) + (-1)^n (n+\tfrac{1}{2}) \pi A_1(t)] \cos (n+\tfrac{1}{2}) \pi x + \\ + 2 \sum_{n=0}^{\infty} e^{-(n+\frac{1}{2})^2 \pi^2 t} \int_0^1 K(\xi) \cos (n+\tfrac{1}{2}) \pi \xi \cos (n+\tfrac{1}{2}) \pi x \, d\xi, \end{aligned} \right\} (79)$$

qui s'écrit avec la notation des fonctions ϑ_2:

$$\left. \begin{aligned} & U(x, t) = -\vartheta_2 (\tfrac{1}{2} x, t) * B_0(t) + \\ & + \frac{\partial \vartheta_2 (\frac{1}{2} - \frac{1}{2} x, t)}{\partial x} * A_1(t) + \tfrac{1}{2} \int_0^1 \left[\vartheta_2 \left(\frac{x-\xi}{2}, t \right) + \vartheta_2 \left(\frac{x+\xi}{2}, t \right) \right] K(\xi) \, d\xi. \end{aligned} \right\} (80)$$

Les mêmes remarques que dans le paragraphe précédent s'appliquent à cette formule.

§ 9. *Conducteur infini d'un côté avec température initiale et radiation à l'extrémité donnée.*

Considérons enfin un problème d'une barre infinie:

$$\frac{\partial^2 U}{\partial x^2} = \frac{\partial U}{\partial t}, \quad \ldots \quad (81)$$

$$\lim_{t \to 0} U(x, t) = K(x), \quad \ldots \quad (82)$$

$$\lim_{x \to 0} \frac{\partial U}{\partial x} = B(t). \quad \ldots \quad (83)$$

Effectuons la transformation de LAPLACE par rapport à la variable t et prenons la transformation de FOURIER suivante pour transformer par rapport à x:

$$\underset{x}{\mathfrak{F}} U(x, t) = \frac{2}{\pi} \int_0^{\infty} U(\xi, t) \cos \lambda \xi \, d\xi. \quad \ldots \quad (84)$$

C'est l'inverse de l'intégrale des cosinus de FOURIER:

$$U(x, t) = \int_0^\infty [\underset{x}{\mathfrak{F}} U(x, t)] \cos \lambda x \, d\lambda. \quad . \quad . \quad . \quad . \quad . \quad . \quad (85)$$

Utilisant la transformation (84), on trouve le théorème de différentiation:

$$\underset{x}{\mathfrak{F}} \frac{\partial^2 U}{\partial x^2} = -\lambda^2 \underset{x}{\mathfrak{F}} U(x, t) - \frac{2}{\pi} B(t), \quad . \quad . \quad . \quad . \quad . \quad . \quad (86)$$

si l'on admet que U et $\dfrac{\partial U}{\partial x}$ s'annulent à l'infini.

Cette formule et le théorème de différentiation de LAPLACE permettent d'écrire l'équation et la solution transformées:

$$-\lambda^2 \underset{t}{\mathfrak{L}} \underset{x}{\mathfrak{F}} U(x, t) - \frac{2}{\pi} \underset{t}{\mathfrak{L}} B(t) = p \underset{t}{\mathfrak{L}} \underset{x}{\mathfrak{F}} U(x, t) - \underset{x}{\mathfrak{F}} K(x), \quad . \quad (87)$$

$$\underset{t}{\mathfrak{L}} \underset{x}{\mathfrak{F}} U(x, t) = \frac{-\dfrac{2}{\pi} \underset{t}{\mathfrak{L}} B(t) + \underset{x}{\mathfrak{F}} K(x)}{p + \lambda^2}. \quad . \quad . \quad . \quad (88)$$

Par suite la transformée de FOURIER de U devient:

$$\underset{x}{\mathfrak{F}} U(x, t) = -\frac{2}{\pi} e^{-\lambda^2 t} * B(t) + e^{-\lambda^2 t} \underset{x}{\mathfrak{F}} K(x). \quad . \quad . \quad . \quad (89)$$

La solution du problème est donc:

$$U(x,t) = -\frac{2}{\pi} \int_0^\infty e^{-\lambda^2 t} * B(t) \cos \lambda x \, d\lambda + \frac{2}{\pi} \int_0^\infty e^{-\lambda^2 t} d\lambda \int_0^\infty K(\xi) \cos \lambda \xi \cos \lambda x \, d\xi. \quad (90)$$

Cette formule peut être transcrite de la manière suivante, si l'on se sert des fonctions de FOURIER (33):

$$U(x, t) = -\chi(x, t) * B(t) + \tfrac{1}{2} \int_0^\infty [\chi(x-\xi, t) + \chi(x+\xi, t)] K(\xi) \, d\xi. \quad (91)$$

Remarquons enfin que le premier terme de ce résultat a été trouvé par DEFANT et ERTEL (2) dans une question océanographique. En effet le problème des perturbations de la salinité de l'océan, causées par la pluie et de l'égalisation de ces perturbations, est régi par l'équation de FOURIER. Les auteurs se sont servis d'un calcul symbolique simultané, calcul qui équivaut à la méthode des transformations de LAPLACE simultanées par rapport aux variables x et t $(\underset{t}{\mathfrak{L}} \underset{x}{\mathfrak{L}} U(x, t))$. La méthode que nous avons suivie $(\underset{t}{\mathfrak{L}} \underset{x}{\mathfrak{F}} U(x, t))$, utilisant des transformations différentes, mène plus vite au résultat cherché.

BIBLIOGRAPHIE.

1. S. R. DE GROOT, Proc. Nederl. Akad. v. Wet., Amsterdam, **45**, 643 (1942).
2. A. DEFANT et H. ERTEL, Abh. preuss. Akad. Wiss., Math.-naturw. Kl., No. 10 (1939).

Mathematics. — *Sur les fonctions univalentes.* Par A. F. MONNA. (Communicated by Prof. W. VAN DER WOUDE.)

(Communicated at the meeting of September 26, 1942.)

§ 1. Récemment j'ai dérivé une inégalité concernant la mesure harmonique des sous-ensembles de la frontière d'un domaine et je l'ai alors appliquée à la fonction de GREEN et au théorème de KOEBE[1]). Je veux donner ici quelques nouvelles applications.

Rappelons d'abord l'inégalité mentionnée. Soit Ω un domaine simple et simplement connexe dont l'origine $w=0$ n'est pas un point intérieur. Soient (R) le cercle $|w|=R$ et $R\theta(R)$ la mesure linéaire de la partie de (R) intérieure à Ω ou située sur la frontière Σ de Ω. Soit Σ_R^e la somme des parties suivantes de Σ: *a*) la partie de Σ extérieure à ou sur (R), *b*) les parties de Σ intérieures à (R) qui appartiennent à la frontière des sous-domaines connexes de Ω, situés à l'intérieur de (R), ne contenant pas P_0 à l'intérieur et dont la frontière est formée par ces parties de Σ et des parties de (R). Ici, P_0 est un point arbitraire de Ω intérieur à (R). Désignons par $\mu^{P_0}(\Sigma_R^e; \Omega)$ la mesure harmonique par rapport à Ω de Σ_R^e dans P_0. Soit d la distance OP_0. Alors on a pour $R \geqq d$

$$\mu^{P_0}(\Sigma_R^e; \Omega) \leqq e^{-\pi \int_d^R \frac{dt}{t\,\theta(t)}} \quad \ldots \ldots \ldots \quad (1)$$

En particulier, puisque $\theta(t) \leq 2\pi$

$$\mu^{P_0}(\Sigma_R^e; \Omega) \leqq \left(\frac{d}{R}\right)^{\frac{1}{2}}. \quad \ldots \ldots \ldots \quad (2)$$

Il est clair que ces inégalités subsistent quand on entend par Σ_R^e seulement la partie *a*), définie ci-dessus.

§ 2. Soit $F(z)$ régulier et univalent pour $|z|<1$ et quotient de fonctions régulières, bornées. Soit $F(0)=0$ et $F(z) \neq w_0$ pour $|z|<1$. On sait que $\lim_{r\to 1} F(re^{i\vartheta})$ existe alors pour presque tous les ϑ. Cette limite, si elle existe, sera désignée par $F(e^{i\vartheta})$. Soit E_R l'ensemble des valeurs de $\vartheta (0 \leqq \vartheta \leqq 2\pi)$ où l'on a $|F(e^{i\vartheta})-w_0| \geqq R$. Donc

$$E_R = E\,(|F(e^{i\vartheta})-w_0| \geqq R) \quad \ldots \ldots \quad (3)$$

mE_R soit la mesure de E_R. Soit, pour $0<\varrho<1$, $C_{t,\varrho}$ la courbe dans $|z| \leqq \varrho$ définie par

$$|F(z)-w_0| = t. \quad \ldots \ldots \ldots \quad (4)$$

Posons

$$l(t) = \lim_{\varrho\to 1} \int_{C_{t,\varrho}} |F'(z)|\,ds. \quad \ldots \ldots \ldots \quad (5)$$

[1]) A. F. MONNA, Sur quelques inégalités de la théorie des fonctions et leurs généralisations spatiales. Proc. Ned. Akad. v. Wetensch., Amsterdam, **45**, 43—50, 165—168 (1942).

Puisque l'intégrale à droite est une fonction croissante de ϱ qui est $\leqq 2\pi t$, cette limite existe.

Je veux démontrer qu'on a pour $|R| \geqq |w_0|$

$$m\,E_R \leqq 2\,\pi\, e^{-\pi \int\limits_{|w_0|}^{R} \frac{dt}{l(t)}} \quad \ldots\ldots\ldots\ldots \quad (6)$$

La démonstration va en deux pas.

A. Supposons $F(z)$ continu dans $|z| < 1$, les limites sur $|z| = 1$ étant différent l'un de l'autre en tous les points de $|z| = 1$. $F(z)$ représente alors $|z| < 1$ sur un domaine Ω, ne contenant w_0 et P_∞ pas comme point intérieur, dont la frontière est une courbe de JORDAN. Les courbes C_t (il n'y a pas besoin maintenant à considérer les courbes $C_{t,\varrho}$) sont représentées sur des arcs du cercle de centre w_0, et de rayon t et avec E_t correspond la partie de la frontière Σ de Ω située hors ou située sur ce cercle; désignons la par Σ_t^e.

En vertu de la formule du § 1 on a

$$\left.\begin{aligned} \mu^O(\Sigma_R^e;\,\Omega) &\leqq e^{-\pi \int\limits_{|w_0|}^{R} \frac{dt}{t\,\theta(t)}} \\ (R &\geqq |w_0|) \end{aligned}\right\} \quad \ldots\ldots\ldots\ldots \quad (7)$$

$t\,\theta(t)$ est la mesure linéaire de la partie dans Ω ou sur Σ de la circonférence du cercle de centre w_0 et de rayon t, c'est à dire c'est justement $l(t)$ définie ci-dessus.

Considérons maintenant la fonction inverse de $F(z)$ qui représente Ω sur le cercle-unité: $z = \Phi(w)$. On a

$$\Phi(w) = e^{-(G+iH)} \quad \ldots\ldots\ldots\ldots \quad (8)$$

où G désigne la fonction de GREEN de Ω avec pôle dans $w = 0$ et H est une fonction harmonique conjuguée de G. Les courbes $H =$ constante forment un système de courbes, partant de $w = 0$ et aboutissant tous en des points bien définies de Σ; ce sont les images des rayons du cercle-unité. L'angle des courbes $H = C_1$ et $H = C_2$ en $w = 0$ vaut $C_2 - C_1$. Soit maintenant σ un arc de Σ. On démontre alors que la mesure harmonique de σ en $w = 0$ est égale à l'angle en $w = 0$ des deux courbes $H =$ constante, aboutissant dans les deux points limitant σ, divisée par 2π [1]).

Appliquons cette propriété à la formule (1). Soient $\alpha_1, \alpha_2, \ldots$ les points d'intersection consécutifs de Σ et du cercle de centre w_0 et de rayon R (remarquons que Σ est une courbe de JORDAN). Σ_R^e est donc composé d'un nombre fini ou d'une infinité dénombrable d'arcs $\sigma_1, \sigma_2, \ldots$. On a

$$\mu^O(\sigma_i;\,\Omega) = \frac{\varphi_i}{2\pi},$$

où φ_i désigne l'angle des deux courbes $H = C$ qui passent par les points α_j, α_{j+1}, limitant σ_i. C'est donc aussi l'angle des deux rayons du cercle-unité qui sont les images de ces deux courbes en faisant la représentation conforme de Ω sur $|z| < 1$ telle que $w = 0$ vient dans $z = 0$. Enfin, φ_i est donc égale à la longueur de l'image de σ_i sur $|z| = 1$. Puisque la mesure harmonique est une fonction d'ensemble complètement additive, on obtient donc par une sommation

$$\mu^O(\Sigma_R^e;\,\Omega) = \frac{1}{2\pi}\, m\,E_R.$$

---

[1]) Voir R. NEVANLINNA, Eindeutige analytische Funktionen (Berlin 1936) p. 28—33.

Donc avec (7)

$$mE_R \leqq 2\pi e^{-\pi \int\limits_{|w_0|}^{R} \frac{dt}{l(t)}}$$

$$(R \geqq |w_0|).$$

B. Passons au cas général.

La limite $\lim\limits_{r \to 1} F(re^{i\vartheta})$ existe presque partout sur $|z| = 1$, disons pour l'ensemble E_0 de valeurs de ϑ. Selon un théorème de EGOROFF il existe un ensemble fermé $E_0' \subset E_0$ où la convergence est uniforme. Soit $\eta > 0$. On a alors pour $r \geqq 1 - \triangle(\eta)$ sur E_0'

$$|F(re^{i\vartheta}) - F(e^{i\vartheta})| \leqq \eta.$$

$$(\triangle(\eta) > 0;\ \vartheta \text{ dans } E_0').$$

Considérons le sous-ensemble E_R' de E_0' où l'on a

$$|F(e^{i\vartheta}) - w_0| \geqq R$$

$$(R > \eta).$$

Alors

$$\begin{aligned} |F(re^{i\vartheta}) - w_0| &= |F(re^{i\vartheta}) - F(e^{i\vartheta}) + F(e^{i\vartheta}) - w_0| \geqq \\ &\geqq |F(e^{i\vartheta}) - w_0| - |F(re^{i\vartheta}) - F(e^{i\vartheta})| \geqq R - \eta \end{aligned}$$

pour $r \geqq 1 - \triangle(\eta)$ et ϑ sur E_R'.

Supposons inversement qu'on a pour une valeur de ϑ et pour $r \geqq 1 - \triangle(\eta)$

$$|F(re^{i\vartheta}) - w_0| \geqq R - \eta.$$

On peut supposer d'ailleurs que $F(e^{i\vartheta})$ existe pour cette valeur de ϑ puisque la limite existe presque partout. Il s'en suit

$$|F(e^{i\vartheta}) - w_0| \geqq R - \eta.$$

On a donc pour $r \geqq 1 - \triangle(\eta)$

$$\left.\begin{aligned} E'(|F(e^{i\vartheta}) - w_0| \geqq R) &\subseteqq E'(|F(re^{i\vartheta}) - w_0| \geqq \\ &\geqq R - \eta) \subseteqq E'(|F(e^{i\vartheta}) - w_0| \geqq R - \eta). \end{aligned}\right\} . \quad (9)$$

Appliquons le résultat *A* au cercle $|z| \leqq r$; puisque F est univalent, l'image de $|z| = r$ est une courbe de JORDAN. Donc

$$mE'(|F(re^{i\vartheta}) - w_0| \geqq R - \eta) \leqq 2\pi r e^{-\pi \int\limits_{|w_0|}^{R-\eta} \frac{dt}{l_r(t)}}.$$

Faisons alors $\eta \to 0$ et donc $r \to 1$. Il résulte de (9)

$$\lim mE'(|F(re^{i\vartheta}) - w_0| \geqq R - \eta) = mE'(|F(e^{i\vartheta}) - w_0| \geqq R).$$

Alors

$$mE'(|F(e^{i\vartheta}) - w_0| \geqq R) \leqq 2\pi e^{-\pi \int\limits_{|w_0|}^{R} \frac{dt}{l(t)}}.$$

De plus en vertu du théorème d'EGOROFF on peut choisir E'_0 tel que sa mesure diffère aussi peu que l'on veut de celle de E_0. Donc

$$m E_R \leqq 2\pi e^{-\pi \int\limits_{|w_0|}^{R} \frac{dt}{l(t)}}.$$

Le théorème est alors démontré.

Remarquons que la borne inférieure des valeurs de R pour lesquelles cette inégalité est valable, est la plus petite si w_0 est sur Σ à plus petite distance de $w = 0$.

Considérons un cas spécial. Supposons que $F(z)$ ne prend pas les valeurs intérieures à un angle φ de sommet w_0. Alors on a $l(t) \leqq (2\pi - \varphi)t$. Donc

$$m E_R \leqq 2\pi e^{-\frac{\pi}{2\pi-\varphi} \int\limits_{|w_0|}^{R} \frac{dt}{t}}$$

et alors

$$m E_R \leqq 2\pi \left| \frac{w_0}{R} \right|^{\frac{\pi}{2\pi-\varphi}} \quad \ldots \ldots \ldots \quad (10)$$

Remarque.

Il est remarquable, que le théorème donne une borne pour la mesure de l'image de la partie de Σ extérieure au cercle $|w - w_0| = R$, tandis que cette borne elle-même ne dépend que de l'allure de Ω intérieur à ce cercle. C'est une conséquence de la propriété suivante de la mesure harmonique: *la mesure $\mu^O(\alpha; \Omega)$ croit lorsqu'on fait croître Ω tel que α reste sur la frontière et $w = 0$ reste un point intérieur. En représentant Ω sur le cercle-unité on obtient: la longueur de l'image de l'arc α croit en faisant croître Ω comme ci-dessus.*

§ 3. Le théorème précédent permet d'obtenir dans certains cas une amélioration du lemme suivant de LÖWNER:

Soit $|w(z)| < 1$ pour $|z| < 1$ et $w(0) = 0$. Supposons que $w(z)$ est continu sur un arc α de $|z| = 1$ et que w y prend des valeurs sur $|w| = 1$. Alors la longueur de l'image $\bar{\alpha}$ de α vaut au moins la longueur de α et l'on n'a l'égalité que si $w = e^{i\vartheta} z$.

Nous supposons ici de plus que $w(z)$ est univalent, de sorte que $w(z)$ représente $|z| < 1$ sur un domaine simple Ω contenu dans $|w| < 1$. La frontière Σ contient un arc $\bar{\alpha}$ de $|w| = 1$. Soit A le point de $\bar{\alpha}$ également distant des points B et C limitant $\bar{\alpha}$ et traçons le rayon OA. Soit w_0 un point non de Ω sur le prolongement de OA. Considérons le cercle (R) de centre w_0 et de rayon R qui passe par B et C. Nous pouvons alors appliquer le théorème si $\bar{\alpha}$ est extérieur à (R) et si $w = 0$ est un point intérieur à (R). Les conditions pour qu'il en soit ainsi s'expriment par

$$\left.\begin{aligned} 1 + 2\,|w_0| \cos \tfrac{1}{2}\psi &> 0 \\ |w_0| + \cos \tfrac{1}{2}\psi &> 0 \end{aligned}\right\} \quad \ldots \ldots \quad \begin{aligned}(11a)\\(11b)\end{aligned}$$

en désignant par ψ l'angle BOC. Supposant ces inégalités vérifiées, on obtient

$$m\,\alpha \leqq 2\pi e^{-\pi \int\limits_{|w_0|}^{R} \frac{dt}{l(t)}} \quad \ldots \ldots \ldots \quad (12)$$

D'autre part on a en vertu du lemme de LÖWNER

$$m\,\alpha \leqq \psi.$$

Une amélioration du lemme est donc obtenue si l'on peut satisfaire à (11*a*), (11*b*) et à

$$2\pi e^{-\pi \int_{|w_0|}^{R} \frac{dt}{l(t)}} \leqq \psi. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (13)$$

(13) se réduit à

$$\int_{|w_0|}^{R} \frac{dt}{l(t)} \geqq \frac{1}{\pi} \log \frac{2\pi}{\psi}. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

Les conditions (11) sont triviales si $\psi \leqq \pi$; dans ce cas il ne reste que (14). En général il résulte de (11)

$$-\cos \tfrac{1}{2} \psi \leqq \frac{1}{2|w_0|} \text{ et } -\cos \tfrac{1}{2} \psi \leqq |w_0|$$

donc

$$\cos^2 \tfrac{1}{2} \psi \leqq \tfrac{1}{2}, \text{ d'où } \psi \leqq \frac{3\pi}{4}.$$

On peut donner à (14) une forme plus facile, quoique moins exacte, en remarquant que $l(t) \leqq 2\pi t$. On a

$$\int_{|w_0|}^{R} \frac{dt}{l(t)} \geqq \frac{1}{2\pi} \log \frac{R}{|w_0|}.$$

A fortiori (14) est donc satisfait si

$$\frac{1}{2\pi} \log \frac{R}{|w_0|} \geqq \frac{1}{\pi} \log \frac{2\pi}{\psi},$$

ou

$$|w_0| \leqq \frac{R\psi^2}{4\pi^2}.$$

Donc, puisque $R^2 = 1 + |w_0|^2 + 2|w_0| \cos \frac{1}{2} \psi$,

$$f(|w_0|) \equiv (16\pi^4 - \psi^4)|w_0|^2 - 2|w_0| \psi^4 \cos \tfrac{1}{2} \psi - \psi^4 \leqq 0. \quad . \quad (15)$$

En considérant ψ donné, par exemple $\leqq \pi$, on a donc obtenu une amélioration du lemme de LÖWNER si $|w_0|$ est au plus égal à la racine positive p de $f(|w_0|) = 0$. Il faut donc que sur le prolongement de AO la distance de Σ à $w = 0$ n'est pas trop grande. Exprimé en termes de $w(z)$ cela veut dire: *si aux points de $|z| = 1$ limitant α on a respectivement $\arg w(z) = \vartheta_2$ et $\arg w(z) = \vartheta_1$ $(\vartheta_2 > \vartheta_1)$* [1]*, en parcourant la courbe définie par $\arg w(z) = \dfrac{\vartheta_2 - \vartheta_1}{2} + \pi$ en partant de $z = 0$ jusqu'au premier point d'intersection avec $|z| = 1$, il faut avoir $|w(z)| \leqq p$. Si $\vartheta_2 - \vartheta_1 \leqq \pi$ on trouve de* (12) *par $l(t) \leqq 2\pi t$, en désignant par m_α et $m_{\bar{\alpha}}$ les longueurs de α et $\bar{\alpha}$*

$$\cos \tfrac{1}{2} m_{\bar{\alpha}} \leqq \frac{(16\pi^4 - m_\alpha^4)|w_0|^2 - m_\alpha^4}{2|w_0|\, m_\alpha^4}. \quad . \quad . \quad . \quad . \quad . \quad . \quad (16)$$

---

[1]) On a $\psi = \vartheta_2 - \vartheta_1$, donc p est déterminé dès qu'on connait ϑ_2 et ϑ_1.

§ 4. La méthode précédente peut s'appliquer aussi pour obtenir des bornes supérieures dans la représentation conforme des domaines convexes. On trouve l'inégalité (12) pour chaque arc $\bar{\alpha}$ de Σ qui se trouve tout entier à une seule côté d'une ligne droite passant par $w = 0$. On a dans ce cas $l(t) \leq \pi t$.

Il en résulte, la signification de R étant la même qu'au § 3,

$$m\,\alpha \leqq 2\pi \frac{|w_0|}{R}. \qquad (17)$$

Les théorèmes précédents montrent une analogie avec le théorème suivant de UNKELBACH[1]):

Soit $F(z)$ régulier pour $|z| < R$; $F(0) = 0$; $F(z) \neq 1$ pour $|z| < R$. $w = F(z)$ représente $|z| < R$ sur un domaine convexe Ω. Alors on a, si α désigne l'ensemble des points de $|z| = R$ où $|F(z)| \geq R$,

$$m\,\alpha < 8. \qquad (18)$$

La démonstration repose sur le lemme de LÖWNER et il sera donc possible, en utilisant (16), d'obtenir une amélioration de (18) pour des classes particulières de $F(z)$. Cependant, les conditions qu'il faut imposer à $F(z)$ ne seront pas d'une forme simple, comme il était le cas pour le lemme de LÖWNER au § 3 fin, et il semble difficile de les obtenir. Il suit de la démonstration de UNKELBACH qu'on peut dire en général que la mesure de l'intersection de la frontière Σ et le cercle $|z| = \varrho$ ne doit pas être trop petit pour $\varrho > R$.

§ 5. L'inégalité (2) permet d'obtenir une inégalité pour la module $|\Phi(w)| = e^{-G}$ de la fonction (8). Choisissons le centre des cercles (R) dans un point de Σ à plus petite distance de $w = 0$; sans nuire la généralité, nous pouvons supposer que c'est le point -1. On a alors $\mu^O(\Sigma_R^e) \leq R^{-\frac{1}{2}}$. On a

$$G(w, 0) = \log \frac{1}{|w|} - g(w, 0).$$

où $g(w, 0)$ désigne la fonction harmonique dans Ω correspondant à des valeurs-frontière $\log \frac{1}{|w|}$. Donc ($P \equiv w$; Q point frontière)

$$g = \int_{\Sigma} \log \frac{1}{PQ}\, d\mu^O(Q). \qquad (19)$$

Divisons cette intégrale en deux parties, l'une I_e étendue sur la partie de Σ extérieure au cercle $|w| = 1$, l'autre I_e, étendue sur la partie intérieure. Puisque $0 \leq \mu^O \leq 1$, on a

$$I_i \geqq \log \frac{1}{1 + |w + 1|}. \qquad (20)$$

Pour I_e on a[2])

$$I_e \geqq -\int_1^{\infty} \log \frac{1}{R + |1 + w|}\, dR^{-\frac{1}{2}},$$

donc

$$I_e \geqq -\log\left[1 + |1 + w|\right] - \frac{2}{\sqrt{|1+w|}} \operatorname{arc\,tg} \sqrt{|1+w|}.$$

---

[1]) H. UNKELBACH, Ueber den Vorrat analytischer Funktionen an groszen Funktionswerten. Jahresber. Deutschen Math. Verein. **49**, 38—49 (1939).

[2]) Voir note 1) p. 48 de mon article mentionné ci-dessus.

Il en résulte

$$g(w, 0) \geqq -2 \log [1 + |1 + w|] - \frac{2}{\sqrt{|1 + w|}} \operatorname{arc} tg \sqrt{|1 + w|},$$

et alors avec $G = \log \frac{1}{|w|} - g$,

$$G \leqq \log \frac{[1 + |1 + w|]^2}{|w|} + \frac{2}{\sqrt{|1 + w|}} \operatorname{arc} tg \sqrt{|1 + w|}.$$

Donc

$$|\Phi(w)| \geqq \frac{|w|}{[1 + |1 + w|]^2} e^{-\frac{2}{\sqrt{|1 + w|}} \operatorname{arc} tg \sqrt{|1 + w|}}.$$

Den Haag, août 1942.

Mathematics. — *Sur une démonstration simple du théorème de déformation de* KOEBE, *et d'un théorème du type* CARLEMAN–MILLOUX. Par H. BOLDER. (Communicated by Prof. W. VAN DER WOUDE.)

(Communicated at the meeting of September 26, 1942.)

Notations et définitions. Indiquons par

Ω: un domaine ouvert simple (schlicht) dans le plan de la variable complexe w, contenant dans son intérieur le point $w=0$, et non le point $w=\infty$, et pour lequel la fonction de GREEN existe;

$C\Omega$: l'ensemble complémentaire de Ω;

Σ: la frontière de Ω;

d: la plus petite distance de 0 à Σ;

$G(0, w)$: dans $\Omega+\Sigma$ la fonction de GREEN, avec pôle en 0, et $=0$ dans $C\Omega-\Sigma$;

$g(w)$: $G(0, w)-\log|w|^{-1}$ pour $w \neq 0$, et continue pour $w=0$;

$\Omega^*, C\Omega^*$ etc: des entités analogues aux précédentes;

domaine de KOEBE (Schlitzgebiet): un Ω, dont Σ se compose des points $w=|w|e^{i\theta}$, où θ est constant, et $|w| \geqq d > 0$. Le point $T=d\,e^{i\theta}$ s'appelle le sommet du domaine de KOEBE.

Introduction. Par la fonction

$$w=f(z)=a_1 z+a_2 z^2+\ldots$$

holomorphe et simple (schlicht) dans $|z|<1$ le cercle $|z|<1$ est représenté sur un Ω simplement connexe. Désignant par $z=\phi(w)$ la fonction inverse, nous avons

$$z=\Phi(w)=b_1 w+b_2 w^2+\ldots, \text{ avec } a_1 b_1=1,$$

et

$$G(0,w)=\log|w|^{-1}+g(w)=-\log|\Phi(w)|=-\log|w|-\log|b_1+b_2 w+\ldots|,$$

d'où

$$g(0)=-\log|b_1|=\log|a_1|.$$

Ceci nous permet de formuler le théorème de KOEBE comme il suit: [1])

Théorème I. *Soit Ω^* un domaine de* KOEBE; *alors pour tout Ω simplement connexe, pour lequel $d=d^*$, l'inégalité $g(0) \leqq g^*(0)$ est valable (c.à.d. $|a_1| \leqq 4d$).*

Le cas d'égalité ne se présente que si Ω lui-meme est un domaine de KOEBE.

La démonstration suivante montrera de plus:

L'inégalité $g(0) \leqq g^*(0)$ subsiste, si la condition d'être simplement connexe est remplacée par la suivante, évidemment moins rigoureuse:

Il y a un point t de Σ, pour lequel $|t|=d$, tel que tout cercle $|w-t|=r$ rencontre un point w au moins, pour lequel $G(0, w)=0$.

La démonstration se fera à l'aide d'une méthode, utilisée par M. M. BRELOT dans sa solution élégante du problème de CARLEMAN–MILLOUX [2]).

---

[1]) A. F. MONNA: Sur quelques inégalités de la théorie des fonctions et leurs généralisations spatiales. Proc. Ned. Akad. v. Wetensch., Amsterdam, **45**, 43 (1942).

[2]) M. BRELOT: Quelques applications aux fonctions holomorphes de la théorie moderne du potentiel et du problème de DIRICHLET. Bull. Soc. Roy. Sci. Liége **8**, 385—391 (1939).

La dernière partie de cet article contient l'indication d'une démonstration analogue d'un théorème du type CARLEMAN–MILLOUX. On peut concevoir le théorème I comme le cas limite pour $R \to \infty$ de ce nouveau théorème.

Démonstration de I. La fonction $G(0, w)$ peut être interprétée comme potentiel (logarithmique) d'une charge $+1$ en 0 et des charges négatives sur Σ. La partie $\log|w|^{-1}$ est la contribution de la charge en 0, donc la partie $g(w)$ est génerée par les charges sur Σ.

Soit Ω^* le domaine de KOEBE ayant pour sommet le point t de Σ, mentionné ci-dessus.

Indiquons à chaque nombre w les nombres

$$w' = t(1 + |t|^{-1}|w-t|), \text{ et } w'' = t(1-|t|^{-1}|w-t|).$$

Balayons toutes les charges de Σ vers Σ', de manière que la charge $-e(p)$ en p arrive à p' (formulé plus exactement: la charge portée par chaque couronne $r_1 < |w-t| < r_2$ ne change pas). Désignons par $h(w)$ le potentiel de la distribution nouvelle sur Σ^*, et posons $H(0, w) = \log|w|^{-1} + h(w)$.

Des relations $|w' - p'| \leqq |w-p| \leqq |w'' - p'|$ nous obtenons

$$-e(p)\log|w'-p'|^{-1} \leqq -e(p)\log|w-p|^{-1} \leqq -e(p)\log|w''-p'|^{-1}, \quad (1)$$

donc, en intégrant par rapport aux charges,

$$h(w') \leqq g(w) \leqq h(w''). \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

et, par addition de $\log|w|^{-1}$,

$$H(0, w') \leqq G(0, w) \leqq H(0, w'') \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

En vertu de (3) et de la condition que $G(0, w)$ s'annule en un point au moins de chaque cercle $|w-t| = r$, nous avons

$$H(0, w) - G^*(0, w) = H(0, w) \leqq 0 \text{ sur } \Sigma^*. \quad . \quad . \quad . \quad . \quad (4)$$

Dans la fonction $H(0, w) - G^*(0, w)$ les singularités ($\log|w|^{-1}$) des deux components se détruisent, et (4) entraîne donc pour tout w

$$H(0, w) \leqq G^*(0, w) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

et

$$h(w) \leqq g^*(w) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (6)$$

(6) se déduit de (5) par soustraction de $\log|w|^{-1}$.

De (3) et (5) nous tirons

$$G(0, w'') \leqq H(0, w'') \leqq G^*(0, w''). \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

et de (2) et (6)

$$g(w'') \leqq h(w'') \leqq g^*(w''). \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (8)$$

(8) contient comme cas particulier

$$g(0) \leqq h(0) \leqq g^*(0) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

$g(w'') = g^*(w'')$ pour $w'' \neq t$ n'est possible, que si toutes les charges, générantes $g(w)$, se trouvent sur Σ^*. Au cas d'un Ω simplement connexe, cela signifie, que Σ et Σ^* coïncident.

Généralisation. Désignons par $L(p, w)$ la fonction de GREEN pour le cercle $|w| < R$, avec pôle en p. Les $L(p, w)$ remplaceront les expressions $\log|w-p|^{-1}$ dans la démon-

stration précédente. Définissons d'une manière nouvelle à chaque w de $|w| \leqq R$ les nombres w' et w'' par

$$w' = t(1+\varrho\,|w-t|), \quad w'' = t(1-\sigma\,|w-t|),$$

où ϱ et σ sont réels et $\geqq 0$, tels que

$$L(t, w') = L(t, w'') = L(t, w).$$

Soit Ω situé dans $|w| \leqq R$, Σ' la partie de Σ située dans l'intérieur $|w| < R$.

Alors il y a une distribution de charges négatives $-e(p)$ *sur* Σ' telle, que nous avons

$$G(0, w) = L(0, w) + \int\limits_{\Sigma'} L(p, w)\, d(-e(p)),$$

dans tout le cerde $|w| \leqq R$.

Balayons encore les charges, de manière que $-e(p)$ arrive à p'.

Au lieu des relations (1) nous avons

$$-e(p)\, L(p', w') \leqslant -e(p)\, L(p, w) \leqslant -e(p)\, L(p', w''). \qquad . \quad (1')$$

Introduisons une nouvelle

$$H(0, w) = L(0, w) + \int\limits_{\Sigma^{*\prime}} L(p', w)\, d(-e(p'))$$

(la distribution de charges est celle après le balayage).

En opérant comme ci-dessus, et songeant que $L(0, w) = \log|w|^{-1} + \log R$, nous obtenons le

Théorème II. *Soit Ω^* la partie d'un domaine de Koebe, située dans $|w| < R, R > d^*$; alors pour tout Ω simplement connexe, situé dans $|w| \leqq R$, pour lequel $d = d^*$, l'inégalité $g(o) \leqq g^*(o)$ est valable.*

Le cas d'égalité ne se présente que si Ω et Ω^* coïncident à une rotation autour de 0 près.

Les relations (7) et (8) restent encore valables, en analogie avec le théorème de Carleman–Milloux.

Nous pouvons formuler II en termes de la théorie des fonctions comme il suit:

Soit $w = f^*(z) = a^*_1 z + a^*_2 z^2 + \ldots$ la fonction, qui représente le cercle $|z| < 1$ sur Ω^*, et soit, dans $|z| < 1$, $w = f(z) = a_1 z + a_2 z^2 + \ldots$ une fonction simple et holomorphe, et $|f(z)| < R$.

Soit Ω l'image de $|z| < 1$ par la représentation $w = f(z)$, et soit $d = d^*$.

Alors on a $|a_1| \leqq |a_1^*|$

$$(\text{c. à. d. } |a_1| \leqslant 4d\,[1 + d\,.\,R^{-1}]^{-2}).$$

Le cas d'égalité ne se présente que si

$$f(z) = e^{i\theta} f^*(e^{i\theta'} z), \ \theta \text{ et } \theta' \text{ réels.}$$

Mathematics. — *Conformal differential geometry.* III. *Surfaces in three-dimensional space.* By J. HAANTJES. (Communicated by Prof. W. VAN DER WOUDE.)

(Communicated at the meeting of September 26, 1942.)

Introduction.

In dealing with conformal differential geometry of surfaces in conformal euclidean spaces various methods can be used. In most of the textbooks about this subject the theory is based upon the isomorphism between the three-dimensional conformal group and a subgroup of the projective group in four dimensions [1]). The coordinates employed are the so called pentaspherical coordinates and a surface is considered as an envelope of a two-parameter family of spheres.

The purpose of this paper is to develop the differential geometry by another method based upon the theorem [2]) that the conformal invariant properties in a flat space are those properties, which are unaffected by a conformal transformation of the fundamental tensor

$$g'_{hi} = \sigma^2 g_{hi}, \quad \ldots \ldots \ldots \quad (1)$$

σ satisfying the equation

$$\partial_j s_i - s_j s_i + \tfrac{1}{2} g_{ji} s_h s^h = 0, \qquad (s_i = \partial_i \log \sigma). \quad \ldots \quad (2)$$

In using this theorem we avoid the introduction of pentaspherical coordinates. Moreover it appears to be unnecessary to look upon a surface as an envelope of a system of ∞^2 spheres.

§ 1. *The conformal invariant fundamental tensors.*

Let x^h $(h, i, j, \ldots = 1, 2, 3)$ be rectangular cartesian coordinates in a three-dimensional flat space R_3, in which the fundamental tensor is denoted by g_{hi}. The equations of a surface S may be written

$$x^h = x^h(u^\alpha); \qquad (\alpha, \beta, \ldots = 1, 2). \quad \ldots \ldots \quad (3)$$

By means of the unit vector n^h, normal to the surface, and the quantity $B^h_\alpha = \partial_\alpha x^h$ the ordinary first and second fundamental tensor are defined as follows:

$$a_{\alpha\beta} = g_{ij} B^i_\alpha B^j_\beta = \sum_i B^i_\alpha B^i_\beta, \quad \ldots \ldots \quad (4)$$

$$h_{\alpha\beta} = n_h \partial_\alpha B^h_\beta = - B^h_\beta \partial_\alpha n_h. \quad \ldots \ldots \quad (5)$$

These tensors are not conformal invariant. From (1) and (4) it follows immediately that

---

[1]) Comp. W. BLASCHKE, Vorlesungen über Differentialgeometrie III, Springer, Berlin, 1929.

T. TAKASU, Differentialgeometrien in den Kugelräumen I, Tokyo, 1938.

[2]) Comp. f. i. J. HAANTJES, Conformal differential geometry I, II, Proc. Ned. Akad. v. Wetensch., Amsterdam, **44**, 814—824 (1941); **45**, 249—255 (1942), referred to as C.D.G. I, II.

the transformation of the first fundamental tensor under conformal transformations (1) is given by

$$a'_{\alpha\beta} = \sigma^2\, a_{\alpha\beta}. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \qquad (6)$$

In order to find the transformation of $h_{\alpha\beta}$ we have to take into consideration that the coordinate system (h) is not a cartesian one with respect to the fundamental tensor g'_{hi}. But if in (5) the ordinary derivative is replaced by the covariant derivative belonging to the fundamental tensor considered the equation will hold for any coordinate system. The parameters of the covariant derivative belonging to g'_{hi} are given by the CHRISTOFFEL symbols constructed with this tensor:

$$\left\{ {h \atop j\,i} \right\}' = A_i^h\, s_j + A_j^h\, s_i - g_{ij}\, s^h, \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \qquad (7)$$

where the unit affinor A_i^h and s_i are defined by

$$A_i^h = 0 \;\; (h \neq i); \;\; A_i^h = 1 \;\; (h = i); \;\; s_i = \partial_i \log \sigma. \quad . \quad . \quad . \quad . \qquad (8)$$

Thus if g'_{hi} is considered as fundamental tensor in R_3, the second fundamental tensor of the surface is given by

$$\begin{aligned} h'_{\alpha\beta} &= - B_\beta^h\, \delta'_\alpha\, n'_h = - B_\beta^h\, \delta'_\alpha\, \sigma n_h = - \sigma B_\beta^h \left(\partial_\alpha\, n_h - B_\alpha^j \left\{ {i \atop j\,h} \right\}' n_i \right) \\ &= \sigma\, (h_{\alpha\beta} - a_{\alpha\beta}\, s); \qquad s = s_i\, n^i. \end{aligned} \quad . \quad . \qquad (9)$$

From (6) and (9) it follows that the normal curvature $\varkappa$ of a given direction transforms under conformal transformations as follows

$$\varkappa' = \sigma^{-1} (\varkappa - s). \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \qquad (10)$$

But since the principal directions at a point are conformal invariant as a consequence of (6) and (9) the transformation of the principal curvatures too is given by (10). Hence the transformations of the mean curvature l and the scalar ϱ, defined by

$$l = \tfrac{1}{2} (\varkappa_1 + \varkappa_2); \quad \varrho = \tfrac{1}{2}\, |\varkappa_1 - \varkappa_2| \quad . \quad . \quad . \quad . \quad . \quad . \qquad (11)$$

are given by

$$l' = \sigma^{-1} (l - s); \quad \varrho' = \sigma^{-1} \varrho. \text{[3]} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \qquad (12)$$

The quantity ϱ vanishes if $\varkappa_1$ and $\varkappa_2$ are equal, which happens only at an umbilical point. In the following umbilical points will be excluded from our considerations. As a consequence of this we have to exclude the case that the surface S is a sphere.

The scalar ϱ enables us to define on the surface a conformal invariant fundamental tensor G_{hi} together with its induced tensor $A_{\alpha\beta}$

$$G_{hi} = \varrho^2\, g_{hi}; \, A_{\alpha\beta} = G_{hi}\, B_\alpha^h\, B_\beta^i = \varrho^2\, a_{\alpha\beta}. \quad . \quad . \quad . \quad . \quad . \qquad (13)$$

The tensor $A_{\alpha\beta}$ is called the *first conformal invariant fundamental tensor* of S.

We shall adopt the convention that a conformal invariant quantity will be denoted by a *capital letter*. The raising and lowering of a suffix of a conformal invariant quantity will be done by means of G_{hi}, $A_{\alpha\beta}$ and the inverse tensors G^{hi} and $A^{\alpha\beta}$. For other

---

[3]) In the following σ is supposed to be positive.

quantities the raising or lowering of a suffix proceeds by means of g_{hi} and $a_{\alpha\beta}$ [4]).

The tensors G_{hi} and $A_{\alpha\beta}$ may be used to introduce several conformal invariant notions. We have for example

a) the conformal invariant "length" of a curve $u^\alpha = u^\alpha(t)$ upon the surface, defined by

$$\tau(t) = \int_{t_0}^{t} \sqrt{A_{\alpha\beta} \frac{du^\alpha}{dt} \frac{du^\beta}{dt}}\, dt; \quad \ldots \ldots \quad (14)$$

b) the conformal invariant normal vector N^h, unit vector with respect to G_{hi} (compare footnote [4])).

c) the conformal invariant derivative of affinors of the surface. This covariant derivative is defined by the equation

$$\nabla_\gamma A_{\alpha\beta} = 0 \quad \ldots \ldots \quad (15)$$

Its parameters $\Gamma^\alpha_{\gamma\beta}$ are therefore the CHRISTOFFEL symbols constructed with the tensor $A_{\alpha\beta}$. From (13) it follows that

$$\Gamma^\alpha_{\gamma\beta} = \left\{ {\alpha \atop \gamma\beta} \right\} + q_\gamma A^\alpha_\beta + q_\beta A^\alpha_\gamma - a_{\beta\gamma} q^\alpha; \qquad (q_\beta = \partial_\beta \log \sigma), \quad \ldots \quad (16)$$

where $\left\{ {\alpha \atop \gamma\beta} \right\}$ are the CHRISTOFFEL symbols belonging to $a_{\alpha\beta}$.

Because of (15) the process of raising and lowering of suffixes by means of $A_{\alpha\beta}$ is commutative with the process of covariant differentiation.

The covariant derivative in R_3. A conformal invariant derivative of affinors in R_3 can be defined if we have at our disposal a quantity q_i with the transformation

$$q'_i = q_i - s_i. \quad \ldots \ldots \quad (17)$$

Then the parameters of this covariant derivative are given by

$$\Gamma^h_{ji} = \left\{ {h \atop j\,i} \right\} + q_j A^h_i + q_i A^h_j - g_{ij} q^h, \quad \ldots \ldots \quad (18)$$

where $\left\{ {h \atop j\,i} \right\}$ are the CHRISTOFFEL symbols constructed with g_{hi}. These CHRISTOFFEL symbols vanish with respect to the system (h), but in writting $\left\{ {h \atop j\,i} \right\}$ on the right hand side of (18), the equation (18) will hold for any other coordinate system and in this form the conformal invariance of the parameters Γ^h_{ji} is at once clear.

From (12) it follows that the q_i, defined by

$$q_i = l\, n_i + q_\alpha B^\alpha_i, \quad \ldots \ldots \quad (19)$$

transforms in the right way. It should be remarked that q_i is a function of u^α, which

---

[4]) Is the unit normal vector with respect to G_{hi} denoted by N^h we have f.i.

$$N_i = G_{hi} N^h; \; n_i = g_{ih} n^h; \; N_i = \varrho\, n_i; \; N^h = \varrho^{-1} n^h.$$

Other conformal invariant quantities are B^h_α and $B^\alpha_i = a^{\alpha\beta} B^h_\beta g_{hi} = A^{\alpha\beta} B^h_\beta G_{hi}$.

means that Γ^h_{ji} is only defined on the surface S. In the following we use the covariant derivative defined by (18) and (19). It will be denoted by the symbol ∇.

The covariant derivative of G_{hi} along the surface appears to be

$$B^j_\alpha \nabla_j G_{hi} \equiv \partial_\alpha G_{hi} - B^j_\alpha \Gamma^l_{jh} G_{li} - B^j_\alpha \Gamma^l_{ji} G_{hl} = 0 \quad . \quad . \quad (20)$$

as follows at once from (18). Hence it is immaterial whether a suffix is raised or lowered by means of G_{hi} before of after the covariant differentiation.

The covariant derivative in R_3 induces in S a covariant derivative with the parameters [5])

$$\Gamma^h_{ji} B^j_\gamma B^i_\beta B^\alpha_h + B^\alpha_h \partial_\gamma B^h_\beta . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (21)$$

These parameters are however identical with $\Gamma^\alpha_{\gamma\beta}$ as may be seen from (16) and (18). This is the reason why we use the same symbol ∇ for both the covariant derivatives in R_3 and on S.

The conformal geodesics. A curve, for which

$$\int d\tau = \int \sqrt{A_{\alpha\beta}\, du^\alpha\, du^\beta} . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (22)$$

is stationary, is called a conformal geodesic. The differential equations for these curves are

$$\frac{d^2 u^\alpha}{d\tau^2} + \Gamma^\alpha_{\gamma\beta} \frac{du^\gamma}{d\tau} \frac{du^\beta}{d\tau} = 0. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (23)$$

In a following paper we shall define the geodesics geometrically.

The second conformal invariant fundamental tensor. This tensor $H_{\alpha\beta}$ is defined by the following equation

$$H_{\alpha\beta} = N_h \nabla_\alpha B^h_\beta = - B^h_\beta \nabla_\alpha N_h = - B^h_\beta B^i_\alpha \nabla_i N_h . \quad . \quad . \quad (24)$$

In comparing this definition with that of $h_{\beta\alpha}$ (formel (5)) it may be proved, that $H_{\alpha\beta}$ is equal to

$$H_{\alpha\beta} = \varrho\, (h_{\alpha\beta} - l\, a_{\alpha\beta}). \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (25)$$

From equation (24) we obtain the following:

$$\nabla_\beta B^h_\alpha = H_{\beta\alpha} N^h \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (26)$$

$$\nabla_\alpha N^h = - H_\alpha^{\cdot\beta} B^h_\beta . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (27)$$

In consequence of (25) the tensor $H_{\alpha\beta}$ satisfies a few algebraic equations. We have

$$\left.\begin{array}{ll} a) & H_\alpha^{\cdot\alpha} = H_{\alpha\beta} A^{\alpha\beta} = 0 \\ b) & Det\,(H_{\alpha\beta}) = - Det\,(A_{\alpha\beta}) \equiv - \mathfrak{A} \\ c) & H_{\alpha\gamma} H^\gamma_{\cdot\beta} = A_{\alpha\beta}. \end{array}\right\} \quad . \quad . \quad . \quad . \quad (28)$$

Since the determinant of $H_{\alpha\beta}$ is negative the two directions defined by

$$H_{\alpha\beta}\, du^\alpha\, du^\beta = 0 \, . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (29)$$

---

[5]) Comp. J. A. SCHOUTEN and D. J. STRUIK, Einführung in die neueren Methoden der Differentialgeometrie I, Noordhoff, Groningen 1935, p. 93.

are distinct and real for real surfaces. It is seen from (28a) that these directions are orthogonal.

The lines of curvature. The principal directions at a point are given by the well-known equation

$$h_{\alpha[1}\, a_{2]\beta}\, du^\alpha\, du^\beta = 0. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (30)$$

Because of (13) and (25) this equation may be written in the form

$$H_{\alpha[1}\, A_{2]\beta}\, du^\alpha\, du^\beta = 0, \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (31)$$

from which the conformal invariant character of the principal directions and therefore of the lines of curvature is evident. The equation for the principal directions may be put in still another form, using the bivector $I^{\alpha\beta}$ defined by

$$I^{12} = -I^{21} = \mathfrak{A}^{-\frac{1}{2}}. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (32)$$

Equation (31) is namely identical with

$$C_{\alpha\beta}\, du^\alpha\, du^\beta = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (33)$$

where $C_{\alpha\beta}$ is defined by

$$C_{\alpha\beta} = H_{\alpha\gamma}\, I^{\gamma\delta}\, A_{\delta\beta} = H_{\alpha}^{\cdot\gamma}\, I_{\gamma\beta}. \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (34)$$

Identities. The components of the quantities $A_{\alpha\beta}$, $H_{\alpha\beta}$, $C_{\alpha\beta}$ and $I_{\alpha\beta}$ satisfy a few algebraic equations, which follow immediately from the definitions of the quantities involved. We give here the equations without proof.

$$\left.\begin{array}{llll} a) & C_{\alpha\beta} = C_{\beta\alpha} & d) & H_{\alpha\gamma}\, C^{\gamma}_{\cdot\beta} = I_{\alpha\beta} \\ b) & C_{\alpha\beta}\, A^{\alpha\beta} = 0 & e) & H_{\alpha\beta}\, C^{\alpha\beta} = 0 \\ c) & Det\,(C_{\alpha\beta}) = -\mathfrak{A} & f) & C_{\alpha\gamma}\, C^{\gamma}_{\cdot\beta} = A_{\alpha\beta}. \end{array}\right\} \quad . \quad . \quad . \quad (35)$$

The equation (b) expresses that the lines of curvature form an orthogonal system.

If the unit vectors (with respect to $A_{\alpha\beta}$) in the principal directions are denoted by P^α and Q^α respectively, the quantities $A_{\alpha\beta}$, $H_{\alpha\beta}$, $C_{\alpha\beta}$ and $I_{\alpha\beta}$ may be expressed in terms of P^α and Q^α We have as a consequence of (32) and (41)

$$\left.\begin{array}{ll} A_{\alpha\beta} = P_\alpha\, P_\beta + Q_\alpha\, Q_\beta; & C_{\alpha\beta} = P_\alpha\, Q_\beta + Q_\alpha\, P_\beta \\ H_{\alpha\beta} = P_\alpha\, P_\beta - Q_\alpha\, Q_\beta; & I_{\alpha\beta} = P_\alpha\, Q_\beta - Q_\alpha\, P_\beta \end{array}\right\} \quad . \quad . \quad (36)$$

As the quantities (36) are linear independent every affinor of order 2 can be expressed as a linear form in $A_{\alpha\beta}$, $H_{\alpha\beta}$, $C_{\alpha\beta}$ and $I_{\alpha\beta}$.

§ 2. *Geometrical interpretations of $A_{\alpha\beta}$ and $H_{\alpha\beta}$.*

The null-directions of $H_{\alpha\beta}$. From the equations (28c) and (35e) it follows that

$$\overset{-1}{H}{}^{\alpha\beta}\, C_{\alpha\beta} = H^{\alpha\beta}\, C_{\alpha\beta} = 0, \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (37)$$

which means that the null-directions of $H_{\alpha\beta}$ defined by (35) are conjugate with respect to the principal directions. But we know already that they are orthogonal. So the null-directions of $H_{\alpha\beta}$ are bisecting the angle formed by the lines of curvature [6]).

---

[6]) From this property it follows that $H_{\alpha\beta}$ is proportional to the tensor c_{ij} used by BLASCHKE, l.c. p. 313.

Another geometrical property of the null-directions of $H_{\alpha\beta}$ at the point P is obtained by considering the spheres, which are at P tangent to the surface. The curve of intersection of such a sphere and the surface has a double point at P. It can be shown that the directions of the tangents at P to this curve are given by

$$(h_{\alpha\beta} - \varkappa\, a_{\alpha\beta})\, du^\alpha\, du^\beta = 0, \quad \ldots \ldots \ldots \quad (38)$$

where $\varkappa$ is the curvature of the sphere considered. The directions (38) are perpendicular if the curvature $\varkappa$ is equal to the mean curvature of the surface. The sphere, which has this property, is called the *central sphere*. From (25) and (38) it is seen that the directions at P of the curve of intersection of the central sphere and the surface are identical with the null-directions of $H_{\alpha\beta}$ [7]).

It is easily seen that the directions determined by equation (38) coincide (principal directions), if $\varkappa$ is equal to one of the principal curvatures of the surface. The corresponding spheres are called the *curvature spheres*. To each principal direction belongs one curvature sphere.

The quadratic form $A_{\alpha\beta}\, du^\alpha\, du^\beta$. Consider the angle between the central spheres at the points u^α and $u^\alpha + du^\alpha$. If the coordinates of the center of the central sphere are denoted by y^h, we get the following expression for the angle $d\varphi$

$$(d\varphi)^2 = l^2 \Sigma \left(\frac{\partial y^h}{\partial u^\alpha} \frac{\partial y^h}{\partial u^\beta} - \frac{\partial l^{-1}}{\partial u^\alpha} \frac{\partial l^{-1}}{\partial u^\beta} \right) du^\alpha\, du^\beta \,. \quad \ldots \quad (39)$$

which leads to

$$(d\varphi)^2 = H_{\alpha\delta}\, H^{\delta}_{.\beta}\, du^\alpha\, du^\beta = A_{\alpha\beta}\, du^\alpha\, du^\beta. \quad \ldots \ldots \quad (40)$$

This formel gives a geometrical meaning of the quadratic form $A_{\alpha\beta}\, du^\alpha\, du^\beta$ [8]).

---

[7]) BLASCHKE, l.c. p. 313.

[8]) It may be seen from this result that $A_{\alpha\beta}$ is identical with the tensor g_{ij} used by BLASCHKE, l.c. p. 312.

Mathematics. — *On the extension of continuous functions.* By J. DE GROOT. (Communicated by Prof. J. G. VAN DER CORPUT.)

(Communicated at the meeting of September 26, 1942.)

Well-known and important is the following theorem [1]): If R is a normal space and F is a (bounded) continuous real function, defined in all points of a closed subset $A \subset R$, then it is possible to find a (bounded) continuous function F', defined in entire R, which is identical with F in the points of A.

This result may shortly be worded as follows: Any F, defined on a closed set $A \subset R$, may continuously be extended on entire R. The closedness of A is therefore in any case a sufficient condition for the possibility of continuous extension.

As far as I know, it has up till now not yet been investigated, how far this condition is also necessary. Still, this question may in the main features be solved in a simple and elementary way.

We shall prove, that for the special case of metric spaces the closednes of A is also a necessary condition for the possibility of continuous extension of any F; that, on the other hand, this condition is not necessary for normal spaces.

So we come to the following

Theorem. *Let A be any subset of a metric space M. Then and only then any (bounded) continuous real function F, defined on A, may be extended to a (bounded) continuous real function F', defined on entire M, if A is a (in M) closed set.*

Contention. *It is possible to construct a normal space N and a not-closed subset A of N, so that any bounded continuous real function F may be continuously extended to a continuous function F', defined on entire N.*

Problem. The question, if the condition, which we just mentioned, is or is not necessary in completely normal (or in other special, but not-metric normal) spaces, has not yet been solved.

Proof of the theorem [2]).

The condition is sufficient: known.

The condition is necessary. Let m be a clusterpoint of A in M, not belonging to A. Now we consider on the set $M-m$ a continuous real function F by setting for every point $p \subset M-m$.

$$F(p) = f(\varrho_p)$$

[1]) Compare P. ALEXANDROFF, H. HOPF, Topologie I, Berlin (1935), p. 73—78 (concerning the terminology we shall follow this book). H. HAHN, Reelle Funktionen, Leipzig (1932), p. 255 (here we only find a proof of the theorem for metric spaces). C. CARATHÉODORY, Reelle Funktionen I, Leipzig and Berlin (1939), p. 155 (a proof of the theorem only for an n-dimensional space R_n). For the case of an R_n this theorem has already been proved in the first edition of the last mentioned book (1918), p. 617 (although by means of single integrals).

[2]) A good deal of the definitive wording of this proof I owe to a communication of Dr. J. RIDDER.

whereby ϱ_p denotes the distance from p to m, while $f(\varrho)$ is a, for a positive ϱ, continuous (bounded) function, for which

$$\lim_{\varrho_a \to 0} f(\varrho_a)$$

has no (finite) limit (the ϱ_a are the distances from the points a of A to m). Such a continuous function F, defined i.a. on A, may obviously not be extended to a continuous function, which is defined on entire M.

Now there yet remains the proof of the existence of such functions F. This may, however, very simply be proved in many different ways. If we don't require the boundedness of F, then for example $F(p) = \frac{1}{\varrho_p}$ satisfies our conditions. Suppose secondly F is bounded. It is always possible to find a sequence of points $\{a_i\}$ of A, converging to m, such that $\varrho_{a_k} < \varrho_{a_l}$ for $k > l$. We then define for instance a bounded continuous function $f(\varrho_p)$, which is exactly 1 for $\varrho_{a_1}, \varrho_{a_3}, \varrho_{a_5}$ etc., and exactly 0 for $\varrho_{a_2}, \varrho_{a_4}, \varrho_{a_6}$ etc. $F(p) = f(\varrho_p)$ then gives the F we asked for.

Proof of the contention. We might construct a space N and a subset A, which satisfies the contention. We attain our end more quickly however by using the following well-known theorem [1]):

Given a completely regular space [2]) R, there exists a bicompact HAUSDORFF space $\beta(R)$, such that: 1° R is dense in $\beta(R)$, 2° any bounded continuous function φ, defined on R, may be extended to a continuous function f, defined on $\beta(R)$.

Because every bicompact HAUSDORFF space is a normal space, we attain our end by setting $N = \beta(R)$ and $A = R$.

---

[1]) Compare A. TYCHONOFF, Math. Ann. **102**, 554 a.f. (1930) and E. ČECH, Ann. of Math. II, **38**, 823 a.f. (1937).

[2]) A regular space is called completely regular, if to every point a and to every closed subset A, which does not contain a, there exists a continuous function $f(x)$, defined in the whole space, such that: $f(a) = 0$ and $f(A) = 1$.

Biochemie. — *L'influence des conditions de l'expérience sur le dosage chronométrique de la peroxydase.* Par H. G. DERX. Laboratoires de Unilever, Rotterdam. (Communicated by Prof. L. G. M. BAAS BECKING.)

(Communicated at the meeting of September 26, 1942.)

Récemment nous avons donné dans ces pages (Proceedings Vol. XLV, N° 7, 1942) l'exposé d'une méthode chronométrique pour le dosage de la peroxydase.

Il paraît utile de considérer de plus près les réactions qui s'effectuent entre l'acide ascorbique, la tolidine et le peroxyde d'hydrogène sous l'influence de la peroxydase.

§ 1. *Cinétique de la réaction.*

Dans le sens chimique et stoechiométrique, la réaction, qui s'effectue pendant le dosage, revient à une oxydation de l'acide ascorbique, jointe à la réduction d'une quantité équivalente de peroxyde d'hydrogène. La concentration de l'ortho-tolidine ne change pas pendant la réaction; cette substance peut donc être considérée comme un catalyseur dans le sens classique. D'un point de vue électronique la réaction consiste en le passage de deux électrons de la molécule d'acide ascorbique à une molécule de peroxyde d'hydrogène liée à la peroxydase et activée par celle-ci.

Nous avons déjà démontré qu'on peut remplacer l'acide ascorbique par un autre réducteur quelconque d'un potentiel d'oxydo-réduction assez bas, pourvu que celui-ci n'exerce pas d'action toxique sur la peroxydase.

Parmi les substances examinées de ce point de vue (pyrocatechol, hydroquinone, pyrogallol, para-phénylènediamine, p.N-methylaminophenol, p. oxyphenylglycine, hydrate d'hydrazine et alloxanthine) il n'y avait que l'hydroquinone et l'alloxanthine qui ont été trouvées utilisables au besoin.

Les autres substances montrent des inconvénients divers: Le pyrocatechol, par exemple, se combine avec l'ortho-tolidine en présence d'un oxydant pour donner un colorant violet. Les autres substances ont des propriétés toxiques per se — comme l'hydrazine — ou bien les produits qui se forment pendant leur oxydation se montrent toxiques. Cette toxicité se manifeste en ceci, que quand on double la quantité de la substance réductrice, la durée de la réaction n'est pas doublée, ou à peu près, mais multipliée hors de toute proportion jusqu'à devenir infinie.

Par contre, en employant comme réducteur l'hydroquinone ou bien l'alloxanthine (respectivement l'acide dialurique), nous avons trouvé, qu'avec des quantités équivalentes de ces corps, pourtant si dissemblables d'un point de vue chimique, la durée de la réaction est exactement la même que celle que l'on trouve avec une quantité correspondante d'acide ascorbique. Nous n'avons pas eu l'occasion de mettre à l'épreuve la cysteïne et le glutathion comme réducteurs, mais les expériences de M. JAYLE (9) font prévoir, que ces deux substances ne changeront pas non plus la durée de la réaction.

Tous ces réducteurs ne sont que des sources d'électrons servant à la réduction de la sémiquinone, qui se forme par oxydation de l'ortho-tolidine.

Puisque, grâce à l'acide ascorbique, la concentration de l'ortho-tolidine reste constante pendant la durée de la réaction, ce n'est que la concentration du peroxyde d'hydrogène qui change et l'on pourrait s'attendre à ce que la réaction prenne un cours (pseudo-) monomoléculaire. Ceci doit se manifester quand on exécute une série d'expériences avec des quantités variables, de plus en plus grandes, d'acide ascorbique. Il est facile de calculer, à l'aide de la formule $K = \frac{1}{t} ln \frac{a}{a-x}$, les relations qui doivent exister entre t_{20}, t_{30}, t_{40}, etc., quand x se monte à 20 %, 30 %, 40 % etc. de a. Le tableau suivant donne,

dans la seconde colonne, les valeurs de $\frac{tx}{t_{20}}$ dans le cas d'une réaction monomoléculaire; dans la dernière colonne se trouvent les valeurs de $\frac{tx}{t_{20}}$ pour le cas d'une réaction rectiligne (ordre zéro).

x	$\frac{tx}{t_{20}}$ monomoléc.	$\frac{tx}{t_{20}}$ rectiligne
20 %	1.00	1.00
30 %	1.60	1.50
40 %	2.29	2.00
50 %	3.11	2.50
60 %	4.11	3.00
70 %	5.40	3.50
80 %	7.21	4.00
90 %	10.31	4.50

Il est en effet possible, que c'est *la concentration de l'o-tolidine qui détermine le cours de la réaction ou qui la limite.* Dans ce cas les durées de la réaction seront simplement proportionelles aux quantités d'acide ascorbique introduites, puisque la concentration de l'o-tolidine reste constante.

Les expériences montrent qu'en général ceci n'est pas le cas. Dans la plupart des expériences les valeurs de $\frac{tx}{t_{20}}$ se trouvent situées entre celles de la réaction monomoléculaire et celle de la réaction rectiligne; les valeurs trouvées dépendent des quantités relatives de peroxyde d'hydrogène et d'ortho-tolidine. Aux concentrations d'o-tolidine peu élevées jointes à des concentrations de peroxyde élevées, la réaction montre une tendance à devenir rectiligne; dans le cas de concentrations normales et élevées d'ortho-tolidine et de concentrations très petites de peroxyde, la vitesse de la réaction est limitée par ce dernier corps et la réaction suit un cours pratiquement monomoléculaire.

Il nous paraît d'ailleurs illusoire pour le moment de vouloir exprimer le cours de la réaction dans une formule mathématique, qui devrait en outre tenir compte des faits suivants:

Comme nous le montrerons, l'activité de la peroxydase dépend *de la valeur absolue* de la concentration du H_2O_2 et celle-ci change pendant la réaction. Ensuite il n'y a pas de relation simple entre l'activité de la peroxydase et la concentration du H_2O_2. L'activité montre un „optimum" à des concentrations de H_2O_2, qui dépendent, comme nous avons pu le démontrer, non seulement de la nature de la peroxydase, de sa pureté et de sa concentration, mais également de la concentration du substratum, dans ce cas de celle de l'o-tolidine.

C'est pour ces raisons que nous avons choisi les conditions du dosage telles, que la concentration du peroxyde d'hydrogène ne diminue que de 5 % pendant la réaction. Dans ces circonstances les déviations d'un cours rectiligne de la réaction deviennent négligeables.

§ 2. *Influences diverses.*

L'optimum de concentration du peroxyde d'hydrogène.

On a reconnu depuis longtemps, que l'activité de la peroxydase change avec la concentration du peroxyde d'hydrogène dans le milieu. En augmentant cette concentration l'activité de la peroxydase augmente également jusqu'à une concentration dite „optimum" pour diminuer ensuite à des concentrations plus élevées encore.

Or, si on calcule les concentrations optima trouvées par differents auteurs, on peut établir le tableau suivant.

Source de la peroxydase	Réaction employée	Molarité optima du H_2O_2	Auteur
Raifort	Leucobase du vert de malachite	7.4×10^{-5}	WILLSTÄTTER et WEBER (13)
Pommes de terre	Réactif Na-di	4.1×10^{-3}	GUTHRIE (6)
Raisins	Gaïacol	2.5 à 5×10^{-4}	HUSSEIN et CRUESS (8)
Navets	Leucobase du 2.6. dichloro-phénol-indophénol	1.7×10^{-2}	DIEMAIR et HÄUSSER (2)

Ces écarts de concentration, qui donnent comme concentration optima $7{,}4 \times 10^{-5}$ M. d'une part et $1{,}7 \times 10^{-2}$ M. (c.à.d. 250 fois plus élevées) de l'autre, peuvent apparaître surprenants, mais les études cinétiques de la réaction par MANN (10) ont démontré, que la concentration optima du H_2O_2 dépend de facteurs multiples, comme par exemple du p_h du milieu, de la nature du substratum et de la concentration de celui-ci.

Ainsi, selon MANN, la concentration optima du H_2O_2 se trouve dans les expériences avec la leucobase du vert de malachite à 6×10^{-5} M. à un p_h 4, mais à 2 à 5×10^{-4} M. à un p_h 3, 2, dépendant de la concentration de la leucobase. Pour l'emploi du gaïacol comme substratum le même auteur a trouvé des concentrations optima variant de 2×10^{-2} M. pour les concentrations élevées à 5×10^{-3} M. pour les concentrations faibles.

Dans tous les cas la concentration optima devient plus élevée quand le substratum devient plus concentré, ce qui confirme l'hypothèse de HALDANE (7), que non seulement le peroxyde d'hydrogène, mais également le substratum doit entrer en combinaison avec l'enzyme, dans ce cas avec la peroxydase.

Nos recherches montrent le même phénomène: Si on augmente la quantité d'ortho-toli-dine, la concentration optima du H_2O_2 s'élève également, comme le montre le diagramme No. 1 pour la peroxydase du raifort.

Diagramme N° 1

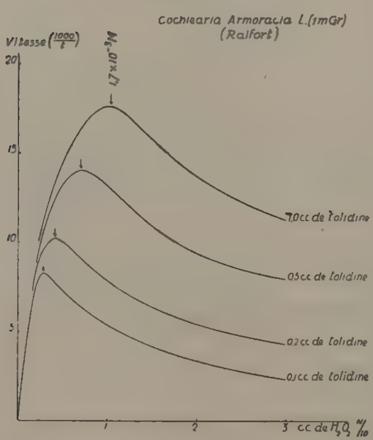
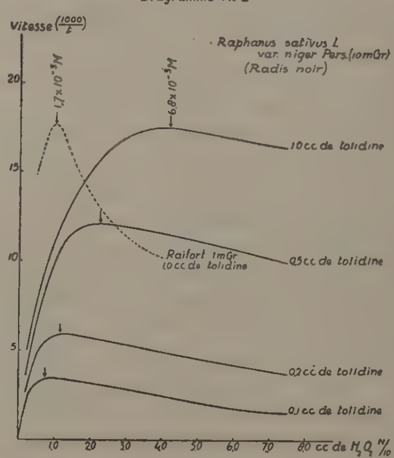


Diagramme N° 2



Le diagramme No. 2 montre en outre un phénomène nouveau. Tandis que la peroxydase du raifort montre une concentration optima de $1{,}7 \times 10^{-3}$ M. de H_2O_2 quand on emploie 1 cc de la solution d'ortho-tolidine à 0,5 %, la peroxydase du radis noir (Raphanus sati-

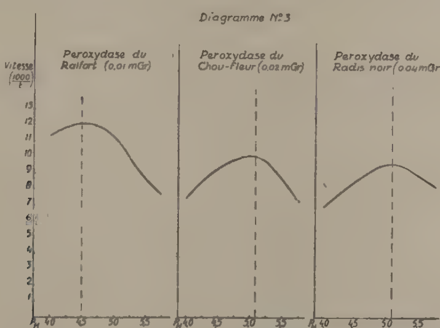
vus L. var. niger Pers.), autre Crucifère, montre dans les mêmes conditions une concentration optima située à $6{,}8 \times 10^{-3}$ M. donc quatre fois plus élevée. La peroxydase du raifort paraît aussi beaucoup plus sensible au changements de concentration du H_2O_2 que la peroxydase du radis noir. Il se peut, que cette dernière soit plus ou moins stabilisée par des substances secondaires, qui se trouvent dans l'extrait (non purifié) et que les différences entre les deux peroxydases s'effaceront à la purification. C'est une étude enzymologique à entreprendre!

Mais nous sommes encore bien loins d'une explication mathématique complète de l'action de la peroxydase.

L'optimum du p_h du milieu de réaction.

Les recherches sur l'optimum du p_h de l'activité de la peroxydase ont montré que celui-ci se trouve aux p_h de 4 à 6. Ce n'est que le pyrogallol qui, employé comme substratum, paraît donner un optimum situé aux environs de p_h 8. Ce p_h élevé paraît bien anormale pour un enzyme végétal, mais il a été confirmé par les études de GETCHELL et WALTON (5) en 1931. La méthode de WILLSTÄTTER et STOLL (12) pour la détermination de la „Purpurogallinzahl" s'effectuant dans de l'eau distillé, c.à.d. dans un milieu non tamponné, doit de ce fait bien manquer de reproductibilité, puisque la réaction s'effectue dans une région ou l'activité est très influencée par le p_h, lequel ne peut pas être bien constant dans les conditions choisies.

Nos recherches sur l'optimum de p_h ont montré, que pour les plantes examinées, l'activité est la plus grande à un p_h de 5,0 à 5,1. Une exception remarquable est fournie par la peroxydase du raifort, qui est la plus active à un p_h de 4,5. La variation de l'activité par le changement du p_h est montrée dans le diagramme No. 3 pour quelques cas typiques.



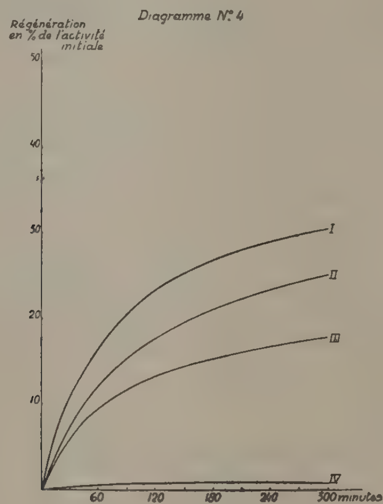
Influence du tampon employé.

Quelques expériences ont été faites dans des milieux dont le p_h 5 avait été établi respectivement par le citrate, l'acétate, le phosphate et le biphtalate. Dans aucune de ces expériences la durée de la réaction n'a été modifiée. Par conséquent, aucune „activation" ne s'est montrée dans ces conditions par le phosphate; il est utile de le noter en vue des expériences de SMIRNOW (11). D'autre part, le choix de l'agent tampon n'est pas indifférent; les tampons au citrate possèdent un avantage supplémentaire, qui n'est pas négligeable: Les extraits végétaux peuvent en effet contenir des traces d'ions ferriques qui, comme on le sait, sont capables de bleuir la benzidine et l'o-tolidine. Ceci est vrai en solution aqueuse par exemple ou bien dans un tampon à l'acétate. Par contre, dans un milieu contenant du citrate, les ions ferriques sont rendus complètement inoffensifs, parceque en présence de l'acide citrique l'ion ferrique donne des complexes, dont le potentiel d'oxydation est trop bas pour causer une oxydation des diamines en question, sans toutefois être assez bas pour pouvoir agir de réducteur.

§ 3. *Régénération partielle de l'activité peroxydasique après l'inactivation thermique.*

Nous allons conclure en relatant quelques observations sur ce curieux phénomène, que nous avons observé dans le courant de nos recherches et que nous ignorions auparavant. Il n'y a en effet que peu de manuels, dont celui de H. VON EULER (3), qui mentionnent le phénomène de la régénération. Celui ci a été étudié pourtant dès 1902/3 par BACH et CHODAT (1) et plus amplement encore en 1924 par GALLAGHER (4). Nous pouvons confirmer tout à fait les expériences du dernier auteur. La simplicité de notre méthode chronométrique, qui permet de déterminer l'activité d'un extrait toutes les 5 ou 10 minutes la rend particulièrement avantageuse pour l'étude de la régénération, phénomène très remarquable, qui mérite plus d'attention qu'il n'a reçu jusqu'ici.

Le diagramme No. 4 donne le résultat d'une série d'expériences avec un extrait de choux de Bruxelles, préparé comme il a été décrite, puis respectivement porté à l'ébullition et refroidi aussitôt après (I), et bouillie pendant 1, 2 et 5 minutes (II, III et IV). Après ce traitement les extraits ont été gardés à la température ambiante. La régénération de l'activité est une fonction hyperbolique ou exponentielle du temps pendant lequel l'extrait a eu l'occasion de se rétablir.



Il nous paraît difficile de se prononcer sur la cause de ce curieux phénomène. On peut penser à une dissociation de la peroxydase à une température élevée, qui séparerait le phéron de l'agon, et à une lente association consécutive de ces deux constituants à la température ambiante. Dans ce cas on doit admettre une thermostabilité relativement grande du phéron; l'agon, c.à.d. la partie hématine de la peroxydase, étant probablement stable aux températures ne dépassant pas 100° C.

BIBLIOGRAPHIE.

1. A. BACH et R. CHODAT, Untersuchungen über die Rolle der Peroxyde in der Chemie der lebenden Zelle. IV. Ueber Peroxydase. Ber. **36**, 600 (1903).
2. W. DIEMAIR et H. HÄUSSER, Zur Kenntnis der Peroxydasen. 1. Ein neues colorimetrisches Bestimmungsverfahren mit 2.6.Dichlorphenol-Indophenol und 1-Ascorbinsäure im Pulfrich Photometer. Z. anal. Chem. **122**, 12—24 et 173—182 (1941).
3. H. V. EULER, Chemie der Enzyme II Teil 3tter Abschnitt. Die Katalasen und die Enzymen der Oxydation und Reduktion. München 1934.
4. P. H. GALLAGHER, Biochem. J. **18**, 39 (1924).

5. R. W. GETCHELL et J. H. WALTON, Some factors influencing the activity of peroxidase. J. Biol. Chem. **91**, 419—433 (1931).
6. J. D. GUTHRIE, A method for the determination of peroxidase activity. J. Am. Chem. Soc., **53**, 242—244 (1931).
7. J. B. S. HALDANE, Enzymes, London 1930.
8. A. A. HUSSEIN et W. V. CRUESS, Properties of the oxidizing enzymes of certain vinifera grapes. Food Research, **5**, 637—648 (1940).
9. M. F. JAYLE, Etude comparative de l'action catalytique des peroxydases végétales et de l'hémoglobine. Bull. de la Soc. de Chimie biologique, Tome **21**, 14—47 (1939).
10. P. J. G. MANN, The kinetics of peroxidase action. Biochem. J., **25**, 918—930 (1931).
11. A. J. SMIRNOW, Biochem. Z. **155**, 1 (1925).
12. R. WILLSTÄTTER et A. STOLL, Ueber Peroxydase. Ann. **416**, 21 (1918).
13. R. WILLSTÄTTER et H. WEBER, Zur quantitativen Bestimmung der Peroxydase. Ann. **449**, 156 (1926).

Petrology. — *Some rocks from the course of the Digoel, the Oewi-Merah and the Eilanden-River (South-New-Guinea).* By W. A. VAN DEN BOLD. (Communicated by Prof. L. RUTTEN.)

(Communicated at the meeting of September 26, 1942.)

The examined material was collected by O. G. HELDRING (1) in 1909, during expeditions of Military exploration-detachments, on pebble-banks in the Digoel, the Oewi-Merah (tributary of the Digoel) and the Eilanden River. It belongs to the collections of the „Mineralogisch-Geologisch Instituut" at Utrecht. The magmatic rocks have been already partly described by E. A. DOUGLAS (2), but it has been proved necessary to revise a number of these determinations.

The knowledge of igneous rocks from S. New-Guinea is very small. C. MOERMAN (3) describes from the region E. of the Etna-bay: Amphibole Diorite, Quartz-Mica Diabase and Amphibole-Mica Andesites (Young effusive rocks). H. TERPSTRA (4) reports as pebbles in the Eilanden-river: Granite, Basalt, Diabase and much Epidote rock. In the Brazza-river (tributary of the Eilanden-river): andesitic dikes with Perlite- and Quartz-dikes. W. J. JONG (5) mentions: 1. Augite Monzonite and Hornblende- and Augite-Hornblende Syenodiorites; 2. Porphyritic Augite Granodiorite; 3. Biotite Granodiorite Porphyry, Augite-Hornblende Granodiorite Porphyry and Hornblende Diorite Porphyry. From the Australian part of the island E. R. STANLEY (7) mentions: Granodiorites, Syenodiorites (Monzonites) and Granite, Diorite and Gabbro; Lamprophyric dikes; Diabases, Porphyries; Augite- and Hornblende Andesites, Olivine Basalt, Trachyte, Rhyolite, porphyritic lavas and volcanic agglomerates. L. AUSTEN (8) only mentions volcanic gravels and effusive rocks without further description from the Fly-River.

Description of rock-samples.

I. *Augite Monzonites* and allied rocks. The most characteristic is an *Augite Monzonite* (514, D23304; 519, D6770; 520, D6771 and D6772) [1] a fine-grained gray rock with some larger patches of Orthoclase and many ore-specks (Magnetite, Pyrite and Chalcopyrite). The rocks show a monzonitic texture: tall idiomorphic Plagioclases and not so beautiful idiomorphic Augite with interstitial Orthoclase. In 519 there occurs also xenomorphic Plagioclase, which possesses sometimes a more acid character than the others. It might also be called a Syenodiorite, just as comparable rocks of the Carstensz Exp. (5). The idiomorphic Plagioclase possesses zonal structure with a core of Labrador-Bytownite and a rim of Oligoclase-Andesine. It often shows broad cracks, filled with Opal. The Augite is yellow green-green-sapgreen pleochroic with often a dark green rim and a nearly colourless core. According to DOUGLAS (2) it is an Aegirine-Augite, but the optical character is always positive. In 520 the axial angle shows tendency to become small; there, a transition to Magnesia-Diopside is present. Sometimes occur many small inclusions of Magnetite in the Augite (514). In 519 the Augite has a much paler colour and it has partly been uralitized; beside this there occurs in 519 a pleochroic Hornblende (nearly colourless-yellowgreen-green). In all the Monzonites there are miarolitic cavities filled with Opal, Chlorite and Calcite. Prehnite and sometimes much pleochroic Epidote (colourless-bright yellow) are secondary minerals. Typical for all these Monzonites and their allies

[1]) The numbers refer to the numbers in the collection of HELDRING, which are also used by DOUGLAS. The D.-numbers refer to the slides. When not specially mentioned, the rocks are from the Digoel.

is the occurrence of large dusty Apatite and very large crystals of Sphene. The dusty crystals of Apatite also occur in two of the dike-rocks (509, 510).

Determination with the integrating stage gave the following result:

520: Plagioclase 42 %, Orthoclase 23 %, Augite 33 %
519: Plagioclase 42 %, Orthoclase 18 %, Mafites 40 %

An *Augite Diorite* (525, D6776) resembles in appearance the Monzonites and some larger patches of felspar proved to be Orthoclase. In the slide, however, no Orthoclase is to be seen. The character of the Plagioclase is the same as in the Monzonites and the Augite resembles the pale-one of 519. It shows alteration into a pale-green Hornblende. Opal also occurs here in cracks. The amount of Sphene is less than in the preceding rocks, and some large dusty Apatite crystals are present. The ores are the same as in the Monzonites. With the Magnetite some brown Biotite is associated.

Augite Granite (516, D6767) is a sugary rock which corresponds with the Monzonites by its large dusty Apatite and its numerous large crystals of Sphene. The texture of the rock is very remarkable: peculiar, cataclastic-looking (but not undulous extinguishing) Quartzes lie in a sort of residual-crystallization of Orthoclase. It most probably has nothing to do with granophyric intergrowth. Next to Orthoclase occur Albite (and Perthite) and Oligoclase. As mafic mineral preponderates a secundary fibrous Hornblende (yellow green-grass green pleochroic) derived from a colourless Diopside. Much Epidote and Zoisite and some Prehnite occur in irregular crystals.

517, D6768, *Gabbro*, resembles in hand-specimen the Augite granite, but contains more dark minerals and ore. It consists of large. xenomorphic, „gabbroidal" Labrador with large cracks, filled with Orthoclase, Chlorite, Epidote and Opal. Colourless Diopside passes into a brown-green Hornblende. There also occurs a primary Hornblende which resembles the secundary. Biotite has been abundant, but it has been altered into Chlorite and Epidote. In this rock also occur large dusty Apatites. Sphene is rare. Epidote-Zoisite occurs in a crush-zone. Prehnite as in 516.

Very different is 526, D6777, an *Amphibole Diorite*, containing Quartz, which occurs as drops in the felspar (xenomorphic Oligoclase-Andesine) which appears to be infiltrated with it. The mafic mineral is a pale (somewhat green-brown pleochroic) Hornblende with often low polarisation colours. Secundary Amphibole occurs in needles. The ore is Pyrite. Sphene is rather abundant in small grains; dusty Apatite occurs in rather thick needles, which are crushed and sometimes bent.

518, D6769 is also described here, although it is not a pure magmatic rock: *Hybrid rock* consisting of *Monzonite* and *Wollastonite-Garnet-Hornfels*. The relationship to the Monzonite is proved by the occurrence of the same typical dark green Augite as in 514 and 520, in irregular aggregates. A basic Plagioclase occurs with Opal in cracks. Quartz is often intergrown with felspar, ranging from Orthoclase to Oligoclase. Accessoria are again the large dusty Apatites and the large Sphenes. Calcite is present in small cavities and distributed in small grains throughout the rock. A further characteristic of this rock is the occurrence of idiomorphic crystals of a brown Melanite. It is found in a zone of the slide in which no Augite is visible. In the other Monzonitic rocks we find no trace of garnet. We consider the garnet to be a contact mineral, as did JONG for the Monzonites of the Carstensz Exp. (5) but we remark, that Melanite may be a primary mineral of Monzonites. The occurrence of Wollastonite equally points to contact-metamorphic processes.

The rocks 514, 518, 519, 520, 525 and a Quartz-Biotite Diorite (513) have been called by DOUGLAS „Nepheline Syenite". H. A. BROUWER has already observed (9), that we have to deal with far more basic rocks and regarded them as „Shonkinitic-theralitic". Our rocks are poorer in Alkali than the Nepheline-Syenites; no Nepheline at all occurs.

Very typical for all these rocks is the association of dusty Apatite and Sphene.

Comparing our material with that of JONG (5) it was stated already, that 519 could be called Syenodiorite. This rock is very much the same as the Hornblende-Augite Syenodiorite D11 and D18 (5). In 519 we observe, that the dusty Apatite grows brighter, while

in JONG's rocks the Apatite is a little dusty in the Syenodiorites and clear in the Monzonites. The Monzonites of both collections differ in more points f.i. the Augite. We can remark here already that also the Hornblende-Diorite Porphyrites (500 etc) are very much the same as the Hornblende Granodiorite Porphyry (D15, DOZY), which contains a little more Orthoclase. As a whole the igneous rocks of the Carstensz Expedition correspond remarkably well with those of HELDRING's collection, though the distance is considerable (at least 300 km).

II. *Hornblende Diorite* etc. These rocks differ from the preceding ones by lack of dusty Apatite (clear Apatite does occur), while Sphene is far less abundant.

Hornblende Diorite (505, D6756 and 6757). This rock passes into a mylonitic breccia, in hand-specimen recognizable as branching green bands in the white and black-green spotted rock. Microscopically it proves to consist of an entirely crushed isotropic mass in which lie splinters of Plagioclase. Near the breccia the texture is foliated. Plagioclase and Hornblende show signs of strain: undulous extinction and fissures. Much Epidote occurs here. The Plagioclases are Andesine-Labrador, others (sometimes not-twinned) are Albite-Oligoclase. The idiomorphic Hornblende is green, strongly pleochroic (yellow-green - dark green) or gray, scarcely pleochroic. The Chlorite with some Epidote has been derived from Biotite. Sometimes it is strongly undulated.

When we consider this rock to be of the same age as the Monzonites a.o. in the vicinity of the „Carstensz Top" it is clear, that during or after the intrusion (Late- or Post Tertiary) movements must have taken place. DOZY observed fractures and mylonites at the contact of the intrusion and the metamorphosed *Lepidocyclina*-Limestone and suggested that these movements were caused to a certain extent by the intrusion itself. DOZY may be right in assuming that no important tectonic movements took place after the intrusion.

Amphibole-Biotite-Granodiorite (507, D6759) is a very light-coloured rock with a typical porphyritical texture: by slight magnification we see at first sight the large hypidiomorphic crystals of Oligoclase and Amphibole in a „matrix" of Orthoclase and Quartz. Larger Orthoclase is also present. The Amphibole is light- dark green pleochroic. Brown Biotite is far less abundant; it passes into Chlorite and Epidote. Accessoria are Apatite, Sphene and Magnetite.

513, D6765 is a fine-grained aplitic *Quartz-Biotite Diorite* consisting for the greater part of an intergrowth of Quartz and Oligoclase-Andesine with some smaller idiomorphic plagioclases (Andesine). The only mafite is brown Biotite in capriceously shaped flakes. Accessoria are Apatite and relatively large grains of Sphene. Calcite is present in miarolitic cavities.

Amphibole Diorite (515, D6766) Digoel and (547, D6782) Oewi-Merah. The first is a dark, fine-grained rock consisting of small xenomorphic Albite and large idiomorphic Labrador (with often zonal structure) and large idiomorphic (lightgreen - browngreen pleochroic) Hornblende. An inclusion of the same rock contains more Pyrite and other ores. 547 differs macroscopically by a much lighter colour. The idiomorphic Hornblendes are to be seen as little bars, stretched into parallel planes. Microscopically there proves to be a second generation of idiomorphic Plagioclase (Oligoclase-Andesine) which also possesses zoning. There are small quantities of Sphene, Apatite, Magnetite, Pyrite, Zircon, Epidote and Chlorite. The cores of the felspars contain Opal.

III. *Porphyritic dikes.* 521, D6773 is connected with the two preceding rocks. It is a *Quartz-Hornblende Diorite Porphyrite* with large fair felspar-phenocrysts (zonal Oligoclase) and small columns of Hornblende. As in 547 three generations of Plagioclase occur. Next to the phenocrysts are idiomorphic crystals of Albite-Oligoclase, while the groundmass consists of Quartz and Albite. The Hornblende is the same as in the Diorites (515 and 547). There is much bright Apatite. The ore is Pyrrhotine.

Other rocks show large white felspar-phenocrysts of Andesine- and Andesine-Oligoclase

in a dark green matrix. The felspars are zonal-built with a more acid rim of Oligoclase. The mafite is an Amphibole in idiomorphic prisms (yellowgreen-darkgreen pleochroic). One of the rocks distinguishes itself by an increasing amount of dark minerals. There is less Quartz in the groundmass, while the others have got a matrix of Quartz, Albite and some Orthoclase (sometimes granophyric intergrowth). Therefore 506, D6758 has been called: *Vintlitic Hornblende Diorite Porphyrite* while the others (500, D23305; 501, D6752; 502, D6753; 503, D6754; 504, D6755; 508, D6760) are called *Quartz-Hornblende Diorite Porphyrite*. They show some resemblance with the Amphibole Diorite (505), but contain much more Quartz and some Orthoclase in the groundmass. Biotite has been present in all these rocks,, but it has been altered into Chlorite and Epidote. Sometimes the felspar has been altered and some Sericite and Calcite occur; Calcite also in miarolitic cavities. Accessoria are much Apatite, sometimes Sphene and Magnetite or Pyrite.

509, D6761 and 510, D6762 show (as 506) Hornblende crystals in a lighter coloured matrix. They, however, don't show much Lamprophyric tendency. They are also called *Quartz-Hornblende Diorite Porphyrite*, and differ from the preceding rocks by the occurrence of exeptionnally large dusty Apatite-crystals.

At last there occur two pebbles of *Quartz-veins* (535 and 536).

IV. *Diabases and allied rocks. Diabase Porphyrite* (511, D6763). It is a grayish-green rock with light green capriceously formed aggregates of felspar-phenocrysts (Albite-Oligoclase), and black-green amygdales. The groundmass consists of Albite-Oligoclase and Pyroxene with ophitic texture. The felspar is sericitized and prehnitized. The pyroxene is a colourless Diopside which sometimes alters into a dusty isotropic mass. The ore is cauliformic Ilmenite. Amygdales of Delessite and Prehnite are present in this rock.

548, D6783 (Oewi-Merah) is perhaps also a *Diabase Porphyrite*, but the phenocrysts have been entirely altered into a saussurite mass in which grains of Epidote and Zoisite occur. The remaining part of the rock possesses an ophitic texture with strongly saussuritized felspar (Andesine or more basic Plagioclase) and colourless Diopside, which passes into a light green Hornblende. The ore is Ilmenite-Leucoxene.

512, D6764, *Diabase*, is a grayish-green, strongly weathered rock with ophitic texture; Diopside in large, sometimes chloritized crystals and lath-shaped Andesine. The ore is Ilmenite-Leucoxene. In some respects this rock resembles 511. Here also occur amygdales of Prehnite.

In the B.-River (Eilanden-River) two *Diabases* have been found, which distinguish themselves by their amount of Quartz, as a residual crystallization. 128, D6446 is a very coarse-grained, 127, D6445 a very fine-grained rock. Both possess a coarse-ophitic texture with large laths of Andesine and Oligoclase, saussuritized (128) or chloritized and silicified (127); Chlorite and Uralite occur, originating from a colourless Pyroxene of which few remnants are present. Accessoria are Apatite and large skeletons of Ilmenite and Leucoxene. DOUGLAS called 127 Diabase, 128 Diorite.

Quartz-Diabases. To these belong three rocks from the B. River. They show a sometimes vague, ophitic texture, and they show a residual crystallization of granophyr. The Plagioclases and Mafites are often idiomorphic.

130, D6448 is a brown-gray, red-spotted rock consisting of completely sericitized, lath-shaped felspars and large Chlorites, in which the form of Augite has been preserved. Filling the interstices there occurs a granophyric intergrowth of Quartz and Orthoclase. Typical for this rock and the two following are long bright Apatite needles.

125, D6443 and 126, D6444 are rather coarse-grained gray rocks. They have been called by DOUGLAS: Quartz-Enstatite Diorite and Quartz Diorite. The Plagioclase is Labrador. Much Magnesia-Diopside has been present, but it has been altered into Uralite in which vermicular remnants of the Diopside still occur. The ore is Magnetite with some Sphene. The residual crystallization is in 125 very finegrained with sometimes myrmekitic

tendency, in the other it is coarse-grained. Here Quartz also occurs alone. The felspar of the granophyr is Albite.

Determination with the integrating stage gave the following result:

130:	Granophyr: 28 %,	Plagioclase: 38 %,	Mafites: 32 %
125:	„ : 21 %,	„ : 45 %,	„ : 33 %
126:	„ : 12 %,	„ : 39 %,	„ : 49 %

For weight percentage this is approximate:

130:	Granophyr: 20–25 %,	Plagioclase: 40 %,	Mafites: 36–40 %
125:	„ : 15–20 %,	„ : 45–50 %,	„ : 35–40 %
126:	„ : 10 %,	„ : 35–40 %,	„ : 50–55 %

TRÖGER gives for Konga-Diabase:

Granophyr: 20 %, Plagioclase: 45 %, Augite: 35 %. For theoretical „Rhyobasalt"
Granophyr: 25 %, Plagioclase: 40 %, Augite: 35 %.

The only real effusive rock from the Digoel is a *Spilite* (524, D6775). It consists of a dark and untransparent glass in which lie felspar-microlites, which are „open" at both sides, with a filling of glass. The felspar has irregular extinction and in most cases proves to be Albite. Chlorite and Prehnite occur in the felspar and in vesicules.

V. Contact-metamorphic rocks. 514, D23306, D23307 is a very peculiar rock consisting partly of quartzitic sandstone (the quartz being strongly strained) with a cement of Prehnite and another part consisting of a fibrous mineral, resembling Prehnite, but with positive zone-character. Further minerals are some Wollastonite and Epidote. It is a *Quartz-Prehnite-Wollastonite-Epidote-Rock.*

544, D6780: *Chiastolite-Cordierite-Biotite Hornfels.* It is a dark rock in which macroscopically the small squares of Chiastolite are clearly to be seen. They are surrounded by a coal-rim and in the way in which in ordinary Chiastolite occur the coalbands, we find here a structure of quartz inclusions. Cordierite occurs in rounded and hexagonal patches. It shows different stages of pinitization. The matrix of the rock consists of quartz, coal and many brown Biotite flakes.

545, D6781 only differs from the preceding by lack of Chiastolite: *Cordierite-Biotite Hornfels.*

549, D6784 forms a transition between the contact rocks and sandstones. It is a quartzitic coalbearing sandstone from the Oewi-Merah, which passes into a Biotite Hornfels.

532, D23310 is a very pure quartzite. The quartz shows beautiful growing-structures. Further occur only some grains of chert.

SUMMARY.

1. A series of rocks from the Digoel river, the Oewih-Merah and the Eilanden-river (S. New-Guinea) has been described. They belong chiefly to a sequence of abyssal rocks ranging from Augite Granite through Augite Monzonite to Gabbro; their contact rocks are also present.

2. Rocks with clear consanguinity have been described formerly from the Carstensz Mt (S. New-Guinea) and from Southern Papua.

3. It may be supposed that all these rocks are of neogene age; this age having been proved for the Carstensz-Mt-rocks.

4. The magma from which the rocks have been derived has not a pronounced pacific character; it must have some atlantic features.

5. The rocks studied by the author have been formerly described by DOUGLAS: his determinations had to be changed frequently.

LITERATURE.

1. O. G. HELDRING, Verslag over Zuid-Nieuw-Guinea. Jaarb. Mijnw. 1911.
2. E. A. DOUGLAS, Annexes III en IV of (1). Jaarb. Mijnw. 1911.
3. C. MOERMAN, Verslag over een geologische verkenningstocht door het terrein beoosten de Etnabaai. Z.W. Nieuw-Guinea Expeditie 1904/5. Leiden 1908.
4. H. TERPSTRA, Resultaten van een goudexploratie in het stroomgebied van de Lorentz- en de Eilanden-rivier in Ned. N. Guinea. De ingenieur in Ned. Ind. 1939.
5. W. J. JONG, Description of rocksamples, in 6.
6. J. J. DOZY, Geological results of the Carstensz Expedition 1936. Leidsche Geologische Meded. Deel IX 1939.
7. E. R. STANLEY, Geology of Papua. Melbourne 1924.
8. L. AUSTEN, Recent Explorations in the northwest district of Papua. Geographical Journal 1926.
9. H. A. BROUWER, Geologie van de Oostelijke Molukken. Jaarb. Mijnw. 1917.

Zoology. — *The influence of Lithium upon the development of the pond snail, Limnaea stagnalis L.* By CHR. P. RAVEN. (From the Zoological Laboratory, University of Utrecht.) (Communicated by Prof. H. J. JORDAN.)

(Communicated at the meeting of September 26, 1942.)

In 1892 C. HERBST, in his studies on the influence of the chemical composition of the surrounding medium upon development, discovered the remarkable effect of lithium ions upon the development of the sea urchin egg. This effect consists in an exaggeration of vegetative differentiations at the expense of animal structures. The differentiation of the stomodaeum, the apical tuft of cilia and the oral arms of the pluteus is suppressed, the entoderm increases at the expense of the ectoderm, so that the ecto-entodermal boundary is displaced towards the animal side of the embryo. When this change in the relative proportions of ectoderm and entoderm is considerable, the invagination of the entoderm at gastrulation is not possible, as the large archenteron finds no longer place in the reduced ectodermal vesicle. The archenteron is pushed outwards instead of inwards: exogastrulation. With increasing action of Li, the extension of the entoderm at the expense of the ectoderm increases progressively, so that vesiculous embryos, consisting entirely of entoderm, develop. Thus, the action of Li on sea urchin embryos brings about an "entodermization" of the presumptive ectoderm.

The development of Amphibia is modified by lithium, likewise. LEPLAT (1920) obtained cyclopic and anophthalmic embryos of *Rana fusca* by the action of LiCl on the gastrula or blastula. ADELMANN used the same agent in his study of cyclopia in *Amblystoma punctatum* (1934). A further analysis of the effect of lithium on the development of Amphibia was given by LEHMANN (1933—1937). In addition to cyclopia, other malformations were obtained by him. In *Triton alpestris*, the effect of Li varies with the stage of development, in which the embryos are exposed to the agent. Exposition during the earlier stages of gastrulation gives embryos, in which the anterior part of the head and the posterior part of the trunk are normal. The hind part of the head is very abnormal; the median parts of the skull are suppressed, the brain is reduced; the ear vesicles are nearly in contact with another in the median plane of the body: otocephalic malformation. The notochord is absent in the head and the anterior trunk region, the myotomes are fused in the median plane, the medullary tube is abnormal. In embryos exposed to LiCl during later phases of gastrulation, on the contrary, the anterior part of the head shows serious malformations; the mouth cavity is much reduced, the visceral skeleton is defective or absent; the brain and sense organs are seriously disturbed, in many cases there is cyclopia: eye and nasal fossa unpaired, prosencephalon much reduced. In addition to this, the notochord is missing in the posterior part of the trunk, with median fusion of the myotomes. On the contrary, the hind part of the head and anterior part of the trunk are nearly normal in this case. With the aid of vital staining, LEHMANN (1937) could show that the loss of the notochord in the embryos treated with Li is not brought about by a destruction of material; only, the differentiation of the notochord is suppressed, the presumptive notochordal cells develop otherwise and become somite cells; in short, the effect of Li results in a "mesodermization" of the notochord material. Probably, the malformations of the neural system and of the sense organs are secondary; they are a consequence of the disturbances in the roof of the archenteron, brought about by the action of Li.

In view of these interesting results of the action of Li on sea urchin and Amphibian eggs, it seemed worth while to study its influence upon other eggs. In the course of our investigations about the development of the pond snail, *Limnaea stagnalis L.*, we subjected

a number of eggs to a treatment with LiCl. The remarkable effects of this treatment induced us to a further analysis.

After some preliminary attempts, solutions of LiCl at a concentration of 0.01 % and 0.001 % in tap water were used. The egg masses were either brought as a whole in the solutions, or they were divided previously into a number of pieces; the individual egg capsules were not isolated from the common jelly mass surrounding them. Both the stage of development, in which the eggs were placed in the solutions, and the duration of the treatment, were varied within wide limits; afterwards, the eggs were returned to tap water and washed thoroughly.

The effects of the treatment fall within two groups. In the first of these, the effects are the most serious; the development is irrevocably disturbed, and comes soon to a standstill. This is the most common of the two. The other group comprises those cases, in which the disturbance is less; development proceeds for a long time, leading to the formation of an embryo, which shows, however, some characteristic malformations.

Embryos of the first group may develop from egg masses, exposed to a wide variety of conditions, as regards concentration employed and length of exposition time. With 0.001 % LiCl, they were obtained from egg-masses placed in the solution immediately after laying and left there for 1, 2 or 3 days; with 0.01 % LiCl, an exposition time of $1^1/_3$—$2^1/_2$ hours is sufficient, provided that the eggs are exposed during the first 6 hours after laying. In the first 2 days of development, the eggs show nothing abnormal. After 3 days, however, when the controls have reached the trochophore stage, the Li-treated eggs show a characteristic appearance: they have grown to large, thin-walled vesicles, filled with a transparent fluid. When one tries to remove them from their capsules, they show a tendency to burst and collapse. The wall consists of two parts, which have a somewhat different appearance; one part is very thin and transparent, and consists of flattened cells, which are nearly free of yolk; the other part is less transparent and often somewhat irregular, its cells are filled with yellow yolk granules (fig. 1). The relationship of these

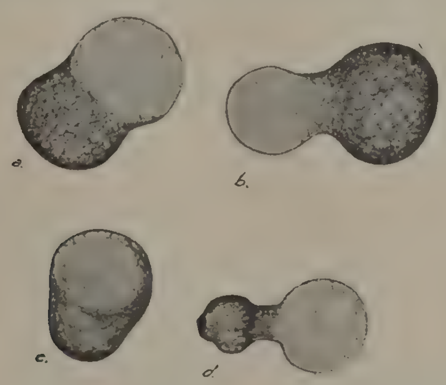


Fig. 1. *Limnaea stagnalis.* Eggs treated with 0.001 % LiCl during 1—2 days. Development of "exogastrulae".

two parts may vary somewhat. In some cases, they are nearly equally large, both composing about a half of the wall; there is often a constriction between the two parts, the embryo being dumb-bell-shaped. In other cases, the yellow part is smaller, the embryo being pear-shaped. Further reduction of this part leads to vesicles, in which the yolk-containing cells are accumulated in a massive heap at one place of the wall; in this case, they may extend into the interior of the vesicle in the form of a dark strand. This last-mentioned category shows a certain resemblance to normal young trochophores, the dark strand looking like a gut. In view of this, it might be allowed to consider the yolk-containing cells as entoderm, the transparent part of the wall as ectoderm. If this be

true, these vesicles would furnish a complete parallel to the exogastrulae of sea urchins. As a rule, these "exogastrulae" show no further development; they may survive for a number of days, but eventually death ensues.

When the eggs are exposed to a solution of 0.001 % LiCl during 2 days, beginning shortly after laying, or to a solution of 0.01 % LiCl during 1 hour immediately after egg deposition, in a number of cases development is disturbed less seriously. At first, it shows nothing abnormal; at the age of 3 days, the trochophore stage is reached. The embryos are, however, smaller than normal controls of the same age, more compact, less transparent, and somewhat irregular in outline. This abnormal appearance increases during the "veliger" stage, and, after some days, it becomes clear that all or most of the embryos of the egg mass show diverse malformations. Usually, a graded series of malformations is obtained, varying from nearly normal young snails to embryos, which are highly teratological. In the lightest cases, the dorsal part of the head is somewhat narrowed, and the eyes, which are placed wide apart in normal embryos (fig. 2 and 3), have approached each other dorsally (fig. 4). This may be accompanied by a splitting of one of the eyes, so that triophthalmic forms arise (fig. 6). In other cases, one of the eyes and tentacles is reduced (fig. 4) or absent: monophthalmia asymmetrica; it is remarkable that in the great majority of cases this reduction process affects the left eye and tentacle.

With increase of the effects of the Li-treatment, the eyes meet each other in the median plane of the head: synophthalmia dorsalis (fig. 5). These cases lead over to the embryos with true cyclopia, where a single eye is situated dorsally in the median plane of the head (fig. 7, 8). Still further reduction of the head region brings about anophthalmic embryos, where the head consists only of a small knob projecting above the foot (fig. 9). Even this may be absent or nearly so: acephalic embryos. It should be noted that the reduction process in all these cases affects the head region, only; the foot and the trunk part of the body with the mantle and shell are normal or only slightly affected. Lastly, there is a group of highly abnormal, teratomorphic forms, in which the relation of the different parts of the body is seriously disturbed.

The physiological aspects of the teratogenetic action of Li have not yet been fully elucidated, in consequence of the complex nature of the problem. The concentration of lithium, the length of exposition time and the stage of development in which the eggs are exposed, determine the effect of the treatment; the interaction of these factors causes a great diversity of the results.

Thus far, it has been made clear that a differential susceptibility of different developmental stages exists. After exposition during 1½ hours to a 0.01 % solution of LiCl in the first 6 hours after laying, all eggs develop into exogastrulae; the same treatment at the blastula stage does not interfere with normal development.

As to the influence of a different length of exposition time, in some cases a remarkable paradoxical effect has been observed, as the following example shows:

6 egg masses (KG—KM) were placed immediately after laying in a 0.001 % solution of LiCl; KG was returned to tap water after 3 hours, KH after 6 hours, KJ 1 day, KK 2 days, KL 2¼ days, KM 3 days. The results of this treatment were: KG and KH normal development to young snails, KJ all eggs to dumb-bell shaped exogastrulae, KK pear-shaped exogastrulae, KL graded series of head reductions: (6 ± normal, 1 synophthalmic, 1 triophthalmic, 3 cyclopic embryos, 1 embryo with monophthalmia asymmetrica, 3 anophthalmic, 5 teratomorphic embryos, 9 †), KM 96 % normal young snails. Thus, with increasing length of exposition time the detrimental effects of the treatment first show a rapid increase, then they decrease again.

Though in some other series a slight indication of the same phenomenon was observed, thus far the effect could not be reproduced. In general, the results of the experiments show a great inconstancy. Partly, this may be accounted for by individual differences in susceptibility of different egg masses. In some instances, where portions of different egg masses of the same age were exposed simultaneously to the same solution during the same length of time, some of them showed a normal development to young snails, while

in others all eggs became exogastrulae. Besides, the influence of external factors, above all the temperature, may account in part for the diversity of the results. Lastly, the procedure of our experiments, in which whole egg masses (or portions of them) were exposed to the solutions, brings into play differences of penetration and adsorbtion of the ions, thereby making things still more complicated. Therefore, a further analysis of the physiological side of the problem will have to work with single egg capsules, freed from the jelly mass. Moreover, the external circumstances, especially the temperature, are to be taken into account. In this way, the relative parts, which concentration of LiCl, length of exposition time, periodical and individual differences in susceptibility play in the causation of the Li effect, may be unraveled.

Summarizing the results, we may say that the influence of Li upon the eggs of *Limnaea* agrees in a large measure with its effects upon the development of sea urchins at one side, of Amphibia at the other. Both exogastrulae, as in sea urchins, and cyclopic or anophthalmic monsters, as in Amphibia, have been produced. If we try to come to an explanation of these facts, it should be remembered that attempts have been made to bring the phenonema observed in Echinoids and Amphibia under a common angle of view.

In sea urchins, the primary influence of Li has been explained as an action upon the gradient-systems of the egg. According to RUNNSTRÖM (1928), development in Echinoids is controlled by the interaction of two gradients, one with high point at the animal, the other with its maximum at the vegetative side of the egg. The Li effects may be explained by a lowering of the animal gradient, leading to a predominance of vegetative differentiations.

Likewise, in Amphibia the effects of Li have been explained from the same point of view. According to DALCQ (1941), Li affects the dorsoventral cortical field, a gradient-field having its high point at the dorsal side of the embryo. Under the influence of Li, the "morphogenetic potential" at this point is lowered, leading to the "mesodermization" of the presumptive notochord material and the inhibition of its inductive action upon the ectoderm, which in its turn brings about the secondary effects upon the neural system and the sense organs. Thus, the primary action of Li would be comparable in both groups. Probably, the animal gradient in Echinoids is localised in the egg cortex, like the dorso-ventral gradient-field of Amphibia. RUNNSTRÖM has observed an ultramicroscopical modification of the cortical layer of the sea urchin egg by the action of Li.

Therefore, the Li effects observed in the eggs of *Limnaea* give support to the hypothesis that the development of the Spiralia is controlled by the interaction of gradient-systems, like those discovered in Echinoids and Amphibia. The action of Li could be explained, then, by an inhibition of one of these gradients, localised, probably, in the egg cortex.

Additional support of this hypothesis may be gained from a comparison of our results with the phenomena observed by CHILD (1915) in Planarians. By the influence of diverse narcotics, head regeneration in *Planaria* may be inhibited; by varying the strength of the agent, a graded series of head reductions is obtained, which shows a complete parallelism to our cases: approach of the eyes at the dorsal side of the head, single median eye, finally complete reduction of the eyes and acephaly. These results are explained by CHILD on the basis of the assumption that the teratogenetical agents interfere with an axial gradient of metabolism, having its high point at the anterior end of the worm.

As noted above, the cyclopic and anophthalmic malformations obtained in Amphibia by the action of Li are, probably, secondary in nature, resulting from the disturbance of the underlying organs by the agent. In Mollusks, we know nothing about an interaction between different organs of the embryo, comparable to the "embryonic inductions" known in Amphibia. Therefore, it is possible that there is only a superficial resemblance between the malformations obtained in both groups, their mode of origin being quite different. However, our experiments make one thing very clear.

According to the common opinion, the eggs of Mollusks belong to the "mosaic eggs". In such eggs, the determination of their parts to different organs of the embryo is fixed at a very early stage of development. Material losses at an early stage cause a defective

development. However, in *Limnaea* eggs treated with Li no loss of cells could be observed. Therefore, the fusion of the eyes to a single median organ in our cyclopic embryos cannot be explained by a fusion of the original lateral eye anlagen, resulting from the loss of the material situated medially to them. We are forced to admit, then, that in our synophthalmic and cyclopic embryos the eyes arise from cells not corresponding to the eye-forming cells of normal embryos; the action of Li has encroached upon the determination process, and brought about an alteration of the plan of the head. In this respect, the cyclopic malformation in *Limnaea* agrees both with that in Amphibia and in Planarians. In one case, from an egg mass kept in tap water until the 8-cell stage, then placed in 0.001 % LiCl for 2 days, a graded series of head reductions, including 6 cases of true cyclopia, was obtained. This proves that at the 8-cell stage the organs of the embryo are not yet definitely laid down. Further analysis will have to show, up to which developmental stage a modification of the plan of the head by Li can be produced.

LITERATURE.

ADELMANN, H. B.: J. exp. Zool. **67**, 217 (1934).
CHILD, C. M.: Individuality in organisms. Chicago 1915.
DALCQ, A.: L'oeuf et son dynamisme organisateur. Paris 1941.
HERBST, C.: Z. wiss. Zool. **55**, 446 (1892).
LEHMANN, F. E.: Die Naturwiss. **21**, 737 (1933).
——————: Verh. Schweiz. Naturf. Ges. **1934**, 360.
——————: Roux' Archiv **136**, 112 (1937).
LEPLAT, G.: Arch. Biol. **30**, 231 (1920).
RUNNSTRÖM, J.: Roux' Archiv **113**, 556 (1928).

Fig. 2.

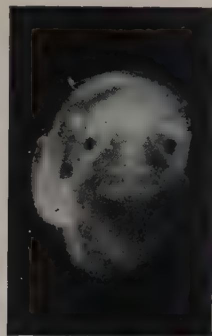


Fig. 3.

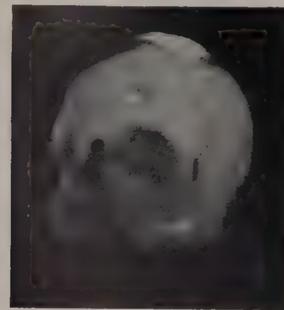


Fig. 4.

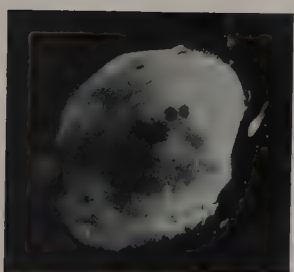


Fig. 5.

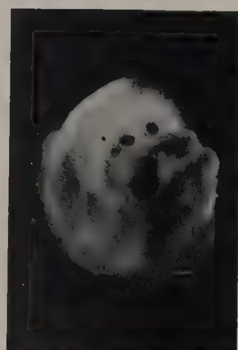


Fig. 6.

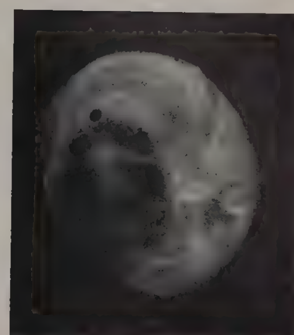


Fig. 7.

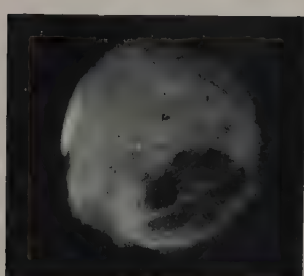


Fig. 8.

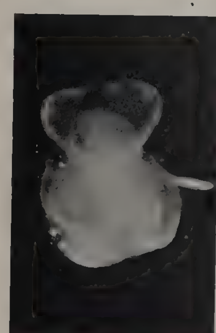


Fig. 9.

Fig. 2—9. Embryos of Limnaea stagnalis.
Fig. 2—3. Normal embryo, 9 days old.
Fig. 4. Synophthalmia dorsalis. Left eye and tentacle reduced.
Fig. 5. Synophthalmia dorsalis.
Fig. 6. Triophthalmia.
Fig. 7—8. Cyclopia.
Fig. 9. Anophthalmia; head region much reduced.

Physiology. — *Over den biologischen grondslag van het handhavende effect van testosteron op de zaadkanaaltjes.* By J. H. GAARENSTROOM and S. E. DE JONGH. (From the pharmacological department, University of Leiden, Holland. Director: Prof. S. E. DE JONGH.) (Communicated by Prof. J. VAN DER HOEVE.)

(Communicated at the meeting of September 26, 1942.)

De door WALSH e.a. ontdekte werking van testosteron (propionaat) op de testes van hyp. looze dieren, t.w. het in stand houden van hun omvang en histologischen bouw, was het onderwerp van een aantal onderzoekingen in ons laboratorium op versch gehypophysectomeerde ratten (GAARENSTROOM en DE JONGH, 1941; GAARENSTROOM, 1941; GAARENSTROOM en DE JONGH 1942).

Wij toonden daarbij aan, dat het effect alleen de testikel*kanaaltjes* betreft en niet het weefsel van LEYDIG. Verder vonden wij, dat het inderdaad tot handhaving beperkt is en dus niet op één lijn te stellen met de werking van bepaalde gonadotrope hormoonpreparaten [1]), afkomstig uit hypophysen, welke de testikelkanaaltjes, en daarmee den geheelen testis, aanzienlijk kunnen doen groeien. Andere gonadotrope preparaten, bijv. die welke afkomstig zijn uit urine van zwangeren, veroorzaken bij de hyp. looze rat uitsluitend een sterke ontwikkeling van het weefsel van LEYDIG (zonder echter merkbaren testisgroei te geven) en dientengevolge productie van veel mannelijk hormoon. Wij maakten aannemelijk, dat de groote ondersteuning, die van deze preparaten uitgaat, wat betreft de groeibevorderende werking op de testikelkanaaltjes van eerstgenoemde, terug te voeren is op de testosteronproductie, die plaatselijk in den testis het peil, dat noodig is voor handhaving van den ontwikkelingsgraad der testikelkanaaltjes, bereikt.

Elk formatief hormoon, gelijk in ons geval de hypophysaire gonadotrope preparaten, vergroot de organen waarop het inwerkt des te moeizamer naarmate zij reeds omvangrijker zijn: hoe grooter zulk een orgaan is, des te belangrijker fractie van de totale hormoondosis noodig is voor het handhaven van den reeds bereikten groei. Bij frequente herhaling van eenzelfde dosis stelt het orgaan zich uiteindelijk in op een bij die dosis behoorenden maximum omvang, waarbij telkens de volle dagdosis voor handhaving wordt gebruikt. In dit licht gezien is begrijpelijk, dat toevoeging van een stof, die deze handhaving kan verzekeren, de taak van een formatief hormoon aanmerkelijk kan ontlasten; de geheele dagdosis van laatstgenoemde stof kan in zoo'n geval groeibevorderend werken.

Uit het bovenstaande blijkt, dat de testikelhandhavende werking van testosteron van beteekenis is voor de *physiologie* (normale ontwikkeling van de testes, onder invloed van lichaamseigen gonadotrope hormonen) en voor de *pharmacotherapie* (vergrooting van klein gebleven testikels met behulp van hormoonpreparaten). Het is dus begrijpelijk, dat wij gezocht hebben naar een aannemelijke verklaring van een verschijnsel, dat bij ons weten in de endocrinologie noch daarbuiten zijn analogon heeft: het groot *houden* van een orgaan zonder de mogelijkheid om het, indien nog klein, groot te *maken*.

Allereerst scheen het zaak dit feit nauwkeurig vast te stellen. Gelijk uit bijgaande tabel I blijkt kan testosteron inderdaad bij ratten van allerlei leeftijd, en dus van uiteenloopend testisgewicht, dit laatste handhaven na hypophysectomie. *Maar een dosis, die een testikel van ca. 1100 mg handhaaft brengt één van 100 mg niet duidelijk tot groeien.*

Somtijds is er een verschil van eenige tientallen milligrammen ten gunste van den tweeden testikel. Dit is uitsluitend het geval bij die dieren (gemerkt met 0) waarbij de

---

[1]) Wij vermijden in dit artikel doelbewust om in te gaan op de theorie van de gonadotrope hormonen. Het is voor ons huidig doel onverschillig, of de hypophyse meer dan één gonadotrope stof met onderling verschillende werkingen afscheidt, dan wel één met meer dan één potentie.

TABEL I. Testisgewicht (mgr.) van hypophyselooze ratten, gedurende 14—28 dagen behandeld met verschillende doses testosteron-propionaat (één testis vóór de behandeling weggenomen).

½ mgr. test. propionaat			1 mgr. test. propionaat			2 mgr. test. propionaat		
Gew. rat (gr.)	vóór behandel.	nà behandel.	Gew. rat (gr.)	vóór behandel.	nà behandel.	Gew. rat (gr.)	vóór behandel.	nà behandel.
69	65[1]	76	54	137[1]	114	80	48[3]	57
74	78[1]	74	61	150[1]	153	80	50[3]	33
52	92[1]	79	71	212[0]	201	68	65[3]	71
51	111[1]	93	80	256[0]	232	79	76[3]	78
48	114[1]	78	73	353[0]	420	78	82[3]	88
49	143[1]	124	70	367[0]	440	85	89[3]	71
50	144[1]	126	56	371[0]	430	99	142[3]	115
58	145[1]	110	57	410[0]	450	69	198[1]	180
55	150[1]	153	55	415[0]	490	72	230[1]	183
64	153[1]	147	85	420[0]	595	74	256[1]	252
59	157[1]	167	101	450[0]	520			
63	233[1]	255	81	500[0]	520			
61	240[1]	222	105	500[0]	550			
67	240[1]	179	72	530[0]	530			
55	261[1]	246	71	560[0]	640			
70	290[1]	234	103	580[0]	690			
152	760[1]*	755	104	670[0]	750			
150	965[1]*	800	173	880[0]	960			
155	1050[1]*	875	179	1120[0]	1030			
145	1060[1]*	600	185	1180[0]	1120			
182	1110[1]*	900						
158	1250[1]*	990						
183	1250[1]*	1050						

[1]) Eén testis (de rechter) weggenomen op den dag van de hypophysectomie, begin der behandeling één dag later.

[0]) Eén testis weggenomen op den dag van de hypophysectomie, begin der behandeling direct daarna.

[3]) Eén testis weggenomen op den 3en dag na hypophysectomie, begin der behandeling direct daarna. De testes van deze dieren waren zeer klein, desondanks reageerden zij op gonadotroop hormoon met sterken groei, het uitblijven van gewichtstoeneming na testosteron-behandeling is dus niet te wijten aan een onmogelijkheid om te groeien.

*) Deze dieren kregen niet 0.5 maar 0.6 mgr. test. propionaat.

behandeling begonnen werd op den dag der hypophysectomie, zoodat wij hechten aan de beteekenis van restanten hypophysehormoon, wier effect door testosteron versterkt wordt. Inderdaad blijft deze groei niet slechts uit bij de dieren (gemerkt met 1) bij wie één dag ligt tusschen het wegnemen van hypophyse + rechter testikel en het begin der inspuitingen, doch ook bij de met 3 aangeduide ratten, waarbij drie dagen na de hyp. ectomie de semicastratie plaats vond en met de inspuitingen werd aangevangen (GAARENSTROOM en DE JONGH (1942).

Ook echter indien men aan de eenige tientallen milligrammen toeneming, die somtijds werden waargenomen, reëele beteekenis wil toekennen, dan treft toch in de allereerste plaats dat een dosis testosteron, die een *grooten* testikel kan handhaven, niet in staat is om een kleinen testis noemenswaard te vergrooten. Behalve in groeipotentie schiet echter het testikelepitheel van hypophyselooze met testosteron behandelde ratten in *niets* te kort; de dieren kunnen zelfs bevruchten. Deze contrasten zijn het, die om opheldering vragen.

Indien wij tegenover het nieuwe verschijnsel van handhaven zonder groeipotentie een objectief standpunt innemen, dan moeten wij twee voor de hand liggende mogelijkheden erkennen. De éene veronderstelt het instellen van alle reeds vóór de hyp. ectomie in gang

zijnde *processen* op een zoodanig tempo, dat een momentopname op elk oogenblik het beeld van vóór de operatie blijft opleveren. Zou b.v. de nieuwvorming van celmateriaal verminderd zijn, dan moet het celversterf daaraan evenredig zijn afgenomen. Testosteron zou dan, oogenschijnlijk toevallig, er steeds juist in slagen om, na de in allerlei fasen van testikelgroei verrichte hyp. ectomie, een toestand te scheppen, waarbij geboorte en sterfte van cellen tegen elkaar zijn uitgebalanceerd. Men kan zich dit echter slechts voorstellen met een voor elken testisomvang afzonderlijke dosis, waarvan de overschrijding groei ten gevolge heeft. De *logica* verzet zich tegen het *dynamisch* beschouwen der handhaving.

De tweede mogelijkheid is, dat de histologische beelden in zooverre bedriegelijk zijn, dat alle na de hypophysectomie schijnbaar voortgaande processen in werkelijkheid zijn gestopt en dat testosteron de atrophie slechts tegenhoudt, door het achterwege blijven van spontaan versterf: het zou dan de *toestand* waarin de operateur den testis achterliet zijn, die door testosteron wordt gehandhaafd. Logisch is hiertegen geen bezwaar. Het geeft echter te denken, dat wij er in slaagden met testosteron testikels, zij het niet geheel volledig, te handhaven gedurende niet minder dan 11 weken (zie tabel II). Ook is het aantreffen

TABEL II. Testisgewicht van hypophyselooze ratten van den dag na hypophysectomie af behandeld met 1 mgr. testosteronpropionaat per dag.

Nummer rat	Gewicht rat (gr.)	Gewicht testis [1]) (mgr.) vóór behandeling		Gewicht testis [1]) (mgr.) nà behandeling		Duur behandeling (dagen)
R 819	185	1180		1120		28
822	173	880	1060	960	1037	28
824	179	1120		1030		28
821	176	1150		900		56
823	175	1180	1062	980	860	56
827	153	855		700		56
825	164	805		820		76
826	162	1105	987	855	832	76
828	164	1050		820		76

[1]) Eén testis (de rechter) werd altijd verwijderd gelijktijdig met de hypophysectomie.

van kerndeelingen, niet slechts, gelijk bij onbehandelde hypophyselooze dieren, in de peripherie, doch ook in de meer centraal gelegen verder gedifferentieerde cellen, een duidelijke aanwijzing, dat de processen *niet* stilstaan. De gedachte aan een *statische* handhaving moet men prijsgeven, tenzij men zou willen denken aan een soort van histologische fixatie tijdens het leven, die ook de mitosen zou omvatten, doch waarmede men zich o.i. zou begeven op het gebied van een *biologische* ongerijmdheid. Na aldus de twee primaire mogelijkheden op logische resp. biologische gronden te hebben verworpen, blijft ons slechts over te zoeken naar een meer ingewikkelde, minder voor de hand liggende verklaringswijze. Gelijk uit het volgende moge blijken, meenen wij er in geslaagd te zijn de eischen van de logica en van de biologie beide te eerbiedigen door den omvang van het door testosteron middellijk of onmiddellijk gehandhaafde *proces*, de spermatogenese, naar boven toe in gedachten te limiteeren door een *toestand*, n.l. den op het moment der hypophysectomie bestaanden omvang van de testikelkanaaltjes.

Wij meenen namelijk een bron voor een hypothese te hebben gevonden in het van zelf sprekende feit, dat groei van de testes (lees: van zijn kanaaltjes) niet wel mogelijk is zonder vermeerdering van het aantal randcellen in die kanaaltjes. De deeling van zulk een randcel geeft aanleiding tot radiaire opschuiving in de bijbehoorende celzuil — die van buiten naar binnen alle overgangen kan vertegenwoordigen tusschen spermatogoniën en rijpe spermiën — doordat één der beide dochtercellen daarin wordt opgenomen. De andere dochtercel erft blijkbaar de deelpotentie der moedercel. Voor groei is noodig, dat *beide* dochtercellen deelpotentie krijgen. Groeibevorderende stoffen (gelijk hypophysaire gonadotrope hormoonpreparaten) *verleenen* dus op een of andere directe of indirecte wijze

een *deel-potentie aan de tweede dochtercel*. Hieruit is af te leiden dat de goed „gehandhaafde" testikel van een met testosteron behandelde hypophyselooze rat een wat de kanaaltjes betreft geheel volwaardig orgaan moet zijn, echter kennelijk zonder het vermogen van de „tweede-dochtercel" om zich te deelen. Daardoor kan het aantal zich deelende spermatogoniën nooit grooter worden dan het tevoren reeds was, of kon zijn, en is groei, voorzoover deze niet bestaat in het grooter worden van cellen, dus uitgesloten. Dit laatste blijkt echter bij histologisch onderzoek niet.

Ten overvloede hebben wij in een groot aantal testikelkanaaltjes van verschillend behandelde ratten tellingen verricht van de randcellen en metingen van de kanaaltjesdoorsnede. De daadwerkelijke structuur der testikels beantwoordt in de practijk niet zoodanig aan de algemeen aanvaarde schemata, dat de verworven getallen geheel betrouwbaar kunnen zijn. Ook moet de lezer zonder bewijsvoering gelooven, dat de gekozen kanaaltjes qua omvang typische vertegenwoordigers waren van de testikels waarin zij werden aangetroffen! Uit het materiaal blijkt desondanks dat de verhouding aantal randcellen : kanaaltjesdoorsnede niet belangrijk schommelt, zoodat het groote verschil in gewicht der testikels op geen stukken na te verklaren kan zijn uit verschil in cel*grootte*. (Zie tabel III.)

TABEL III. Vergelijking tusschen testisgewicht, doorsnede zaadkanaaltjes en aantal randcellen in die kanaaltjes, bij hypophyselooze ratten, behandeld met testosteronpropionaat en hypophysair gonadotroop hormoon.

Aantal ratten	Aanvangsgewicht rat (uitersten) (gr)	Behandeling (gedurende 18 dagen) dagel. dosis	Aantal getelde zaadkanaaltjes per testis	Gem. doorsn. zaadkan.[1]	Gem. aantal randcellen	Gem. testis gew. (mgr.)	
4	84—110	0.2 cc olijfolie	10 10	60 36	79 48	570 129	vóór behandeling nà behandeling
4	101—105	1 mgr. test. propionaat	10 10	59 67	81 81	550 630	vóór behandeling nà behandeling
4	94—111	5 R.E. ambinon[2]	10 10	58 73	77 91	560 919	vóór behandeling nà behandeling

[1]) Relatieve maten.

[2]) Gonadotroop-thyreotroop preparaat uit hypophysen bereid (N.V. Organon, Oss).

Daarentegen bestaat een merkwaardige correlatie tusschen de testikelgrootte en de gem. tubulusdoorsnede, resp. het gem. aantal randcellen gelijk uit het volgende blijkt:

	Contrôle-ratten	Testost. ratten	Ambinonratten
Tub. doorsnede	1	1.9	2.0
Aantal randcellen	1	1.7	1.9
$\sqrt[3]{\ }$ testisgewicht	1	1.7	1.9

Bij al de in tabel III vermelde dieren werd op den dag der hyp. ectomie met de inspuitingen begonnen, zoodat met testosteron een zekere testisgroei mogelijk was. Inderdaad bedragen de gemiddelde gewichten der testes voor en na de behandeling 550 mg en 630 mg. Deze geringe gewichtsvermeerdering van de testis der testosterondieren in deze proef t.o.v. hun eigen vóór de proef uitgenomen contrôletestikel, *kan* teruggevoerd worden tot een verandering in *celgrootte* (gelijkgebleven aantal randcellen bij toegenomen tubulusdoorsnede). Voor de grovere gewichtsverschillen tusschen de testikels der afzonderlijke diergroepen is deze factor kennelijk te verwaarloozen.

Met dit alles zijn de positieve werkingen van testosteron geenszins opgehelderd, *doch het uitblijven van groei biedt geen moeilijkheid meer*. Gaan wij thans wat nader in op de feiten, die om verklaring vragen.

Indien een rat gehypophysectomeerd wordt, valt met de groeibevorderende werking van het hyp. hormoon ook de prikkel tot het maken van testosteron weg. De testikelkanaaltjes houden nu niet slechts op te groeien, doch worden zelfs veel kleiner, en het aantal cellagen van hun inhoud neemt af. Het is duidelijk dat de sterfte van hun cellen

den aanmaak overtreft. De spermatogoniën aan de peripherie gaan echter, gelijk door verschillende onderzoekers, waaronder wijzelf, is opgemerkt, door met zich te deelen. Het aantal mitosen is t.o.v. normale of met hormonen behandelde dieren niet verminderd. Het onderscheid in kanaaltjesgrootte moet dus liggen aan de vergroote sterfte. Aangezien geen redenen bestaan om aan te nemen, dat de laatst ontstane cellen het eerst sterven, zullen de zuilen van binnen naar buiten worden ingekort. Het kleiner worden der kanaaltjesdoorsneden moet dan berusten op een aanpassing van hun omtrek aan den verminderden inhoud.

Hierbij moeten spermatogoniën met deelpotentie van den rand worden weggedrongen, waarop wij straks terugkomen.

De atrophie is dus terug te brengen tot een vermindering van levensvatbaarheid; de opvatting ligt derhalve voor het grijpen, dat *de verhindering dezer atrophie en het hoogblijven der celzuilen door testosteron op een vergrooting van den levensduur der deelingsproducten van de zich ahormonaal deelende spermatogoniën berust.* Doordat alle kanaaltjes hun omvang door testosteron bewaren, blijft het gewicht van den ganschen testikel ten naaste bij constant. Testosteron handhaaft echter niet alleen het testikelgewicht (celzuilen van groote hoogte) het doet ook de spermatogenese voortduren, d.w.z. de metamorphose, die de cellen op elke hoogte van de zuil van elkaar doet verschillen, gaat door. Moet men daartoe een afzonderlijken prikkel, uitgaande van testosteron, aannemen? Uit een oogpunt van redeneering is dat overbodig: het is duidelijk dat een dergelijke herhaalde metamorphose (inclusief reductiedeeling!) tijd kost en slechts plaats kan vinden bij voldoenden levensduur van het celmateriaal. Zijn de zuilen kort, gelijk bij het dier waarbij de hyp. eenigen tijd geleden verwijderd is, dan moet de spermatogenese uitblijven ook al zou de potentie ertoe aanwezig zijn. Zijn de zuilen lang genoeg, dan kan de anders sluimerende potentie zich uiten. Er zijn nu bovendien practische aanwijzingen, dat de spermatogenese ahormonaal verloopt. Zeker weten wij dit van haar allereerste phase, nl. de deeling der spermatogoniën. Vermoeden doen wij het van volgende stadia, aangezien testosteron aan *kleine* hyp. looze testikels geen spermatogenese kan *verschaffen*. Opmerking verdient overigens, dat geen enkele hormoonbehandeling ooit in staat is gebleken *overhaaste* spermatogenese te geven. Niet slechts beneden een bepaalden kanaaltjesomvang blijft spermatogenese uit; ook indien (bij ratten) tengevolge van hormoontoediening een zekere omvang in geforceerd tempo bereikt wordt, blijft de graad van spermatogenese bij dien omvang ten achter (MOORE).

De spermatogenese kan dus in het algemeen, bij voldoenden beschikbaren tijd, opgevat worden als een *functie* van de celzuilhoogte, die geen afzonderlijke hormonale prikkel behoeft. Een voldoende zuilhoogte wordt echter slechts bereikt wanneer eenerzijds de levensduur der cellen groot genoeg is om de producten van eenige achtereenvolgende deelingen tegelijkertijd in leven te doen zijn, anderzijds de doorsnede der kanaaltjes (en dus de omtrek!) groot genoeg is om dergelijke groote zuilen te herbergen!

Er volgt hieruit dat *testosteron*, voor spermatogenese slechts dan toereikend is, indien de benoodigde ruimtelijke verhoudingen (en daarmede de spermatogenese zelf) tevoren reeds bestonden. Is dit niet het geval dan kan de benoodigde groote doorsnede (omtrek) der kanaaltjes slechts verschaft worden door hyp. hormoon. De groei van den testis, welke op den duur een toenemende spermatogenese waarborgt, is dus afhankelijk van twee condities: voldoenden levensduur der cellen en voldoende toeneming van het aantal cellen met deelpotentie. Dat houdt in, dat hypophysehormoon door het verleenen van deelpotentie *alléén* geen groote testikels kan doen ontstaan. De gebruikelijke preparaten hebben echter alle in meerdere of mindere mate ook het vermogen de testes tot testosteronproductie aan te zetten.

Wij missen alle zekerheid, dat een van deze capaciteit bevrijd hypoph. preparaat nog eenigen testisgroei zou kunnen geven. Wel zijn er redenen om te vermoeden dat de zichtbare werkingen van een hyp. preparaat op de testikelkanaaltjes uitermate gering zijn bij armoede aan dat, wat volgens de meeste onderzoekers als een afzonderlijke componente wordt opgevat: het hormoon dat de functie van de cellen van LEYDIG aanzet.

Veronderstellen wij dus, dat testosteron aan de spermatogenetische elementen slechts een langeren levensduur verschaft dan waarover zij spontaan beschikken (ze dus in hun wezen „handhaaft") dan zijn de gevolgen — even zoovele verschillen t.o.v. de aan zijn lot overgelaten hypophyselooze rat — onder meer:

1. De zuilen blijven zoo lang als de, door den omtrek der kanaaltjes bepaalde, beschikbare ruimte toelaat.

2. De omtrek blijft constant, daar de centrifugaal verloopende atrophie uitblijft en dientengevolge elke reden, waarom het aantal randcellen zou verminderen, ontbreekt.

3. De spermatogenese kan (ahormonaal) voort blijven gaan op het bestaande peil.

4. Een beperkte groei van den testis is slechts mogelijk voor zoover de kanaaltjes de door de tevoren aanwezige cellen met deelpotentie geboden mogelijkheden niet zouden hebben uitgebuit.

Dit laatste is het geval indien de rat vóór de hypophysectomie een relatief te kort zou hebben gehad aan de stof, die de cellen van LEYDIG aanzet. Dan was de hoeveelheid beschikbaar testosteron niet in harmonie met de deelpotentie der spermatogoniën; de zuilen zouden relatief kort gebleven zijn, de omtrek zou zich daaraan hebben aangepast; er zouden cellen met deelpotentie, in niet randstandige posities zijn terechtgekomen.

Wij nemen dus aan, dat het maximum *aantal* celzuilen wordt bepaald door het aantal spermatogoniën met deelpotentie, de maximum *hoogte* der zuilen (en daarmee de meest vergevorderde fase der spermatogenese) door den levensduur der testikelelementen. Eerstgenoemd maximum kan echter niet worden bereikt als de zuilhoogte gering blijft, het tweede indien de door den kanaaltjesomtrek gewaarborgde ruimte het niet toelaat. Hoe hooger de zuilen immers trachten te worden, des temeer zitten zij elkaar naar het centrum in den weg en des te nauwkeuriger zullen zij zich rangschikken met den kanaaltjesomtrek als basis. Levensduurverlenging (toediening van meer testosteron dan tevoren) heeft geen groei ten gevolge, indien de rangschikking reeds volledig basaal was.

Bij een dier dat na de hypophysectomie onbehandeld blijft, neemt het aantal cellen met deelpotentie niet langer toe, doch ook niet af. Verder worden de zuilen korter en de inhoud der kanaaltjes geringer, naar wij veronderstellen door verkorting van den levensduur der cellen. De atrofie is dus centrifugaal, want de centrale cellen hebben het langst geleefd en zijn daardoor het meest tot afsterven geneigd. De omtrek past zich mechanisch aan bij den geringen inhoud, *waarbij onvermijdelijk spermatogoniën met deelingspotentie van den rand weggedrongen worden*. Hierdoor wordt de eenige mogelijkheid geschapen voor een *niet onbelangrijken testisgroei door testosteron*: verlengde levensduur der cellen; strekken der zuilen; spannen van den omtrek, doch wel te verstaan niet verder dan tot het op het tijdstip der hypophysectomie bereikte (of bereikbare) maximum.

En inderdaad, juist in *deze* proefopstelling kon NELSON kleine testes met testosteron vergrooten. Hij hypophysectomeerde volwassen ratten en behandelde ze na weken rust. Wij gaven er de voorkeur aan [1]) zeer jeugdige ratten te opereeren en direct te behandelen, gelijk wij boven zagen met als eenig resultaat handhaving van het testisgewicht. Vóór wij de thans beschreven hypothese opstelden erkenden wij tusschen beide proefopstellingen geen wezenlijk verschil. In het licht van onze theoretische beschouwingen is dat onderscheid echter eminent. *Inderdaad slaagden wij er kort geleden in de opgaven van* NELSON *te bevestigen door zijn opstelling nauwkeurig te volgen!* (Zie tabel IV). De groei van de testes was in onze gevallen niet zoo belangrijk als bij NELSON en nauwelijks meer dan bijv. bij de dieren uit tabel III. Doorslaggevend voor ons was echter, dat door het lange wachten elk spoor van invloed der eigen hypophyse met zekerheid uit te sluiten is, in tegenstelling tot de proeven van tabel III, waarbij direct na de operatie werd ingespoten. Drie dagen pauze zou op grond van ons overige materiaal (zie tabel I) voldoende zijn geweest om groei uit te sluiten! Na zoo lang wachten dat een sterke atrophie der testikels

---

[1]) Bij velen is het hypophysectomeeren van *jonge* dieren een moeilijkheid; wij hebben meer last met het lang *in leven houden* van hyp.looze ratten.

TABEL IV. Groei van tevoren *geatrophieerde* testes (door een 17-daagsche pauze tusschen hypophysectomie en behandeling) bij volwassen hypophyselooze ratten, behandeld met testosteronpropionaat.

Gewicht rat (gr.)			Testis gewicht (mgr.)[1]		Behandeling[2] (dagelijksche dosis)
by hypophysect.	vóór behandeling	nà behandeling	vóór behandeling	nà behandeling	
244	193	215	485	545	2½ mgr. test. propion.
238	182	199	335	380	2½ „ „ „
248	202	202	320	380	2½ „ „ „
230	183	183	320	455	2½ „ „ „

[1]) Eén testis (de rechter) werd op den 1en behandelingsdag verwijderd.

[2]) Behandeling gedurende 20 dagen.

ontstaan was, blijkt nu weer groei door testosteron mogelijk. Deze groei is dus van anderen aard dan die uit tabel I en III.

Dat de dosis, in overeenstemming met NELSON 2½ mg per dag bedraagt en dus hooger is dan in onze vroegere proeven (maximum 2 mgr per dag) kan nauwelijks van beteekenis zijn, gezien de lichaamsgewichten der gebruikte ratten, welke veel hooger lagen dan in één van onze vroegere proeven.

De uitkomsten van deze proeven leveren ons niet alleen een aangename bevestiging van NELSON's gegevens en een bevredigende opheldering van gemis aan eenstemmigheid met een alleszins ervaren onderzoeker, doch tevens een argument ten gunste van onze opvatting.

Het blijft ons thans nog over aannemelijk te maken, dat de testikelgroei, die het gevolg is van het toedienen van hypoph. gonadotrope hormoonpreparaten aan hyp. looze ratten, versterkt kan worden door testosteron.

Welnu, genoemde gonadotrope preparaten bevatten een overmaat van de stof, die volgens de in dit artikel gehuldigde opvatting aan pas ontstane spermatogoniën deelingspotentie kan verschaffen, terwijl de stof die via de cellen van LEYDIG testosteronproductie veroorzaakt er relatief weinig in pleegt voor te komen. De niet optimale levensduur der spermatogenetische zuilcellen maakt dus, dat de kanaaltjes niet zoo groot zijn als zij op grond van hun aantal cellen met deelingspotentie konden wezen. Toevoeging van testosteron bovendien moet het groeibevorderend effect van het hyp. preparaat versterken.

Een paar mogelijke bronnen van misverstand dienen nog uit den weg geruimd te worden.

1. Indien wij de werkingen der in dit artikel genoemde hormonen omschrijven als het verleenen van deelpotentie en het verlengen van levensduur, dan worde daarmee niets geantecipeerd met betrekking tot hun werkelijke *aangrijpingspunten*. Wellicht werken de hormonen tendeele bijv. via het vaatstelsel van de testes! Bedoeld is slechts dat het éene hormoon (het gonadotrope) *hoe dan ook* aan nieuwe cellen het vermogen verschaft zich te deelen, naast hun spontane potentie tot metamorphoseeren en dat het andere (testosteron), *onverschillig op welke wijze*, hun levensomstandigheden dusdanig verbetert, dat zij minder vlug sterven.

2. Wanneer in het bovenstaande van bepaalde processen (deeling van spermatogoniën die reeds deelingspotentie bezitten, spermatogenetische metamorphose en bijbehoorende reductie-deeling) wordt aangenomen dat ze „ahormonaal" verloopen, heeft dit in de eerste plaats uitsluitend betrekking op de hier ter sprake gebrachte hormonen. Een eventueele beteekenis van schildklier, bijnier of welke andere klier dan ook voor deze processen kwam niet in discussie. Verder bedoelen wij met den term „ahormonaal" niet meer dan dat wij een hormonalen invloed *voor ons betoog*, dus ter wille van het leveren van een sluitende voorstelling, niet noodig hebben. Het betreft hier dus slechts een geval van denkeconomie. Wij kunnen ons voorstellen dat genoemde hormonen in de toekomst ook nog voor onze beschouwingswijze niet noodzakelijke, doch eventueel daarmede wel vereenigbare, andere werkingen zullen blijken te hebben.

Tenslotte laten wij een schematisch overzicht volgen, dat aan moet duiden, hoe wij ons het beeld van testikels van op verschillende wijze behandelde ratten voorstellen en waarin vele van bovenstaande beschouwingen samengevat tot uiting komen.

I. *Rat na hyp. ectomie; onbehandeld*:

Opschuiving van cellen door deeling van spermatogoniën. Levensduur dezer cellen uiterst beperkt. Tempo afsterven overtreft tempo opschuiving. Centrifugale verkorting der spermatogenetische zuilen en daarmee teruggloopen van den differentiatiegraad der cellen. Aanpassing aan het geringere celmateriaal door omtrekbeperking. Onvermogen om het aantal cellen met deelpotentie te doen toenemen.

Eindtoestand: kleine kanaaltjes, waarin niet alle cellen met deelpotentie randstandig zijn.

II. *Idem, behandeld met testosteron*:

Opschuiving door deeling als boven. Levensduur normaal of maximaal (afh. van dosis). Gelijkblijven in lengte der spermatogenetische zuilen of zelfs verlenging, zoolang de afmetingen van het kanaaltje dit toestaan. Behoud of geringe verbetering van spermatog. differentiatiegraad. Onvermogen om het aantal cellen met deelpotentie te verhoogen; geen vergrooting van eenige beteekenis der kanaaltjes. Eindtoestand: kanaaltjes even groot of iets grooter dan vóór operatie; alle cellen met deelpotentie randstandig.

[III. *a*. Idem, behandeld met de zuivere hypophysaire fractie die het kanaaltjesepitheel prikkelt (theoretisch geval). Nagenoeg als I behoudens vermogen om aantal cellen met deelpotentie te doen toenemen. Uit gebrek aan levensvatbaarheid dezer cellen blijft dit vermogen nagenoeg zonder invloed.

Eindtoestand: kleine kanaaltjes met zeer veel cellen met deelpotentie.]

b. Idem, behandeld met de gebruikelijke hyp. extracten die naast den kanaaltjes aanzettenden factor ook min of meer bevatten van den factor, die via de Leydigsche cellen de testosteronproductie aanzet.

Opschuiving door deeling. Matig behoud van levensduur, waardoor behoud van spermatogenese. Sterke vermeerdering van het aantal cellen met deelpotentie.

Eindtoestand (afhankelijk van relatieve hoeveelheid eigengemaakt testosteron): ongeveer handhaving of zelfs flinken groei der kanaaltjes, met goede spermatogenese.

IV. *Idem, behandeld met testosteron en een gebruikelijk hypoph. extract.*

Opschuiving door deeling. Maximale levensduur en toenemen der cellen met deelpotentie waarborgen tezamen volledig ontwikkelde spermatogenetische zuilen. Alle cellen met deelpotentie randstandig.

Eindtoestand: kanaaltjesomvang maximaal, na groei in maximaal tempo.

Summary.

1. Testosterone is able to maintain the weights of the testes of freshly hyp. ectomized rats, independent of their original weights.
2. Testosterone can only enlarge testicles, when given a few weeks after the hypoph. ectomy, to animals which already had large testes.
3. The testicular weight can be maintained for 11 weeks.
4. On maintenance of the testicle weights through testosterone, as with the growth of the testes through hyp. hormone, the proportion between the diameter of the canals and the number of their circumferential spermatogonia is unchanged.
5. On the ground of these facts and of others, known before a working hypothesis is drawn up of the difference between maintenance and growth of testicles.

LITERATUUR.

E. L. WALSH, W. K. CUYLER en D. R. MC. CULLAGH, Amer. J. Physiol. **107**, 508 (1934).

J. H. GAARENSTROOM, Arch. intern. pharmac. et thér. **66**, 121 (1941).

J. H. GAARENSTROOM en S. E. DE JONGH, Arch. intern. pharmac. et thér. **65**, 206 (1941); Acta brev. neerl. **12**, 73 (1942).

C. R. MOORE, Amer. J. Anat. **59**, 63 (1936).

W. O. NELSON, Anat. Rec. **79**, abstr. 48 (1941).

Medicine. — *Bloedsuikerdaling door Insuline en lichaamsgewicht*. Door S. E. DE JONGH en R. W. SPANHOFF. (Uit het Pharmacologisch laboratorium der R.U. Leiden en het Biologisch laboratorium der N.V. Organon, Oss). (Communicated by Prof. J. VAN DER HOEVE.)

(Communicated at the meeting of September 26, 1942.)

Bij het ijken van insuline, met als criterium de daling van het bloedsuikergehalte van hongerende konijnen, wordt meestal stilzwijgend verondersteld, dat het lichaamsgewicht van tamelijken invloed is op den omvang dier daling en wel in dien zin, dat deze het geringst is bij de zwaarste dieren. Hier en daar heerscht zelfs de gewoonte de in te spuiten dosis evenredig aan het lichaamsgewicht te kiezen. Ook waar men dit niet doet, pleegt men toch bij de verdeeling der dieren over standaard- en onbekend preparaat angstvallig rekening te houden met de gewichten.

Ten einde op dit punt grootere zekerheid te verwerven, hebben wij een gedeelte van de te onzer beschikking staande ijkingsresultaten aan eenige eenvoudige mathematische bewerkingen onderworpen. Het materiaal omvat *alle* (2593) uitkomsten van insuline-inspuitingen in 1939 en de eerste 3 kwartalen van 1940, verricht in het laboratorium der N.V. Organon, voorzoover de volgende voorwaarden vervuld waren:

1. Alleen mannelijke chinchilla konijnen werden meegeteld, wier gem. % bloedsuikerdaling meer dan 10 % bedroeg en wier aanvangswaarde tusschen 0.9 en 1.3 ‰ suiker lag.
2. De dosis bedroeg 0.5 i.E. van eenzelfde preparaat (1 mg = 16.7 i.E.).
3. De konijnen hadden 24 uur gehongerd.

Deze restricties hebben geen andere bedoeling dan om het materiaal homogeen te maken; b.v. *vrouwelijke* konijnen, ingespoten met bijv. $^1/_3$ i.E., na b.v. *twee* dagen hongeren zouden een even goede populatie hebben opgeleverd.

Voor elk der 2593 inspuitingen wordt in het vervolg gebruik gemaakt van drie grootheden.

1. *Het gewicht* van het konijn en wel zoodanig, dat b.v. 2150 beduidt een gewicht tusschen 2100 en 2200 gram.
2. *Het aanvangsbloedsuikergehalte* en wel zoodanig, dat b.v. 1025 beduidt een aanvangsbloedsuiker tusschen 1.000 en 1.050 pro mille.
3. *De som der bloedsuikers* ¾, 1½, 2¾ en 3 uur na de inspuitingen van insuline; het getal 3200 beduidt dan b.v. dat deze som 3.2 ‰ bedroeg.

Alvorens naar wetmatige betrekkingen tusschen de eerste en derde der genoemde grootheden te gaan zoeken, overwogen wij de volgende mogelijke bron van fouten: Het is bekend, dat het aanvangsbloedsuikergehalte niet zonder invloed is op de diepte der daling en dus op de onder 3. beschreven „som". Stel nu, dat er een seizoensschommeling in deze aanvangsbloedsuikers bestond en tevens één (gelijk of tegengesteld gericht) in de gewichten, dan zouden de verschillen in bloedsuikerdaling, die het gevolg zijn van de eerste schommeling, verkeerdelijk kunnen worden toegeschreven aan de tweede. Daarom werden in tabel I de 2593 inspuitingsproeven gerangschikt naar de aanvangsbloedsuikergehalten, en wel telkens voor één kwartaal. De in elk vakje ingevulde getallen duiden dus de frequenties aan, waarmee in een zeker kwartaal een bepaalde aanvangsbloedsuikergehalte werd waargenomen. Van regelmatige seizoensschommelingen blijkt daarbij niets: een geringe verschuiving van het frequentiemaximum naar omlaag vond plaats in het tweede kwartaal van 1939; de eenige duidelijke periode met een verschuiving naar

omhoog is het derde kwartaal van 1940. In een ouder materiaal van konijnen werd overigens destijds evenmin een invloed van het seizoen in den thans bedoelden zin waargenomen [1]).

Tegelijk werd de frequentie-verdeeling over de gewichtsgroepen vervolgd (tabel II).

TABEL I.

Rangschikking der proeven naar de aanvangsbloedsuikergehalten.

Periode	925	975	1025	1075	1125	1175	1225	1275	1325	1375	Som
1e kw. 39	5	45	50	**63**	53	32	25	7	0	0	280
2e „	22	57	**108**	**90**	69	38	30	14	4	4	436
3e „	17	42	81	**88**	**103**	80	57	29	7	5	509
4e „	11	30	62	94	**114**	105	42	11	0	0	469
1e kw. 40	4	12	40	80	57	56	30	6	0	0	285
2e „	4	8	27	65	**68**	66	62	23	0	0	323
3e „	19	26	46	46	**48**	**57**	39	10	0	0	291
Som:	82	220	414	**526**	**512**	434	285	100	11	9	**2593**

Ook hierbij ontbreekt een reproduceerbare seizoensinvloed: in 1939 werden de konijnen geleidelijk iets zwaarder, om in 1940 constant te blijven.

De tabellen I en II ontzenuwen dus het theoretische bezwaar, dat tegen een bewerking van ons materiaal kan worden ingebracht.

TABEL II.

Rangschikking der proeven naar de gewichten der konijnen.

Periode	1750	1850	1950	2050	2150	2250	2350	2450	2550	2650	2750	2850	2950	3050	3150	Som
1e kw. 39	1	8	17	34	40	**44**	38	33	35	21	7	2	0	0	0	280
2e „	6	11	21	46	68	**82**	61	50	40	27	14	6	4	0	0	436
3e „	2	3	8	34	33	55	**78**	67	64	55	56	30	24	0	0	509
4e „	2	2	8	10	22	36	67	**63**	**70**	56	45	35	48	3	2	469
1e kw. 40	0	0	0	2	4	22	28	37	**45**	35	35	27	17	27	6	285
2e „	0	2	3	5	13	26	50	**58**	39	46	31	29	14	1	6	323
3e „	0	2	3	7	10	30	34	47	**50**	37	33	19	14	5	0	291
Som:	11	28	60	138	190	295	356	**355**	343	277	221	148	121	36	14	2593

Ter beantwoording van de hoofdvraag, die wij ons gesteld hadden, werd tabel III samengesteld. Op één *rij* zijn telkens vereenigd alle gegevens van konijnen uit eenzelfde aanvangsbloedsuikergehaltegroep; in één *kolom* die van konijnen uit eenzelfde gewichtsgroep. De aanvangsbloedsuikers nemen toe van boven naar beneden, de gewichten van links naar rechts. In elk *vakje* wordt aangegeven hoeveel waarnemingen erin vallen, benevens het totaal hunner „bloedsuikersommen" van ¾—3 uur.

De *onderste rij* geeft de gemiddelde bloedsuikersom per kolom, onder vermelding van het totaal aantal waarnemingen. Hieruit kan dus afgelezen worden tot hoe laag de bloedsuiker gemiddeld daalde bij dieren met toenemend gewicht.

De *laatste kolom* geeft de gemiddelde bloedsuikersom per rij, wederom onder vermelding van het aantal waarnemingen. Hieruit ziet men hoe laag de bloedsuiker gemiddeld daalde bij dieren met toenemend aanvangsbloedsuikergehalte.

Deze laatste relatie is destijds aan ander materiaal door DE LEEUW [2]) uitvoerig en

---

[1]) R. W. SPANHOFF, Pharmaceutisch Weekblad nr. 23, 1939.

[2]) Acta brevia neerl. 6, 17 (1936).

TABEL III.

Betrekking tusschen aanvangsbloedsuiker, lichaamsgewicht en insulinewerking.

Gew.	1750	1850	1950	2050	2150	2250	2350	2450	2550	2650	2750	2850	2950	3050	3150	gemidd.	(gecorr.)
Aanv. bls. 925	4210 2	2532 1		12955 5	31635 11	37605 13	24645 9	27805 11	38690 14	18645 7	13605 5	4830 2	4595 2			2704 82	
975	2650 1	14710 5	35615 13	63710 23	86835 30	57770 20	130405 47	81550 29	46860 17	30970 11	23390 9	25135 9	9075 3	7975 3		2819 220	(2788)
1025	2410 1	19080 7	26615 10	74585 27	107210 36	176640 59	162780 56	162925 57	150645 54	115235 42	94550 34	50185 17	35735 13	2460 1		2853 414	(2854)
1075	7325 3	14019 5	35960 12	79930 28	121968 41	150049 51	213980 71	217017 74	231240 79	154550 55	129515 44	96570 33	54580 19	28015 9	5600 2	2928 526	(2920)
1125	12359 4	10930 4	40811 13	61468 20	100045 33	168177 56	205063 69	251103 84	198604 67	153858 51	136375 46	80750 28	74610 26	31020 10	3445 1	2986 512	2986
1175		6845 2	16840 6	50700 16	54925 18	135165 44	171345 57	162145 53	176495 58	184390 60	129656 43	89630 29	98045 33	32975 10	14665 5	3050 434	(3052)
1225		5920 2	11713 4	43030 14	52800 16	112134 35	105204 33	95080 30	119025 38	97223 32	88125 28	63280 21	73260 24	9795 3	17150 5	3136 285	(3118)
1275		5530 2	2890 1	12890 4	15405 5	36979 12	37650 12	37742 12	45415 14	57235 18	34426 11	23640 7	2660 1		3000 1	3155 100	(3184)
1325			3100 1	2929 1		8575 3	3000 1	9895 3	3290 1	2360 1						3014 11	
1375						6800 2	3290 1	6010 2	3470 1		3200 1	7012 2				3309 9	
Gemidd.	2633 11	2842 28	2892 60	2941 138	3004 190	3017 295	2972 356	2961 355	2956 343	2940 277	2954 221	2980 148	2914 121	3118 36	3133 14	2593	

nauwkeurig bestudeerd en in een correctiefactor vastgelegd. Met behulp daarvan kan men berekenen tot hoe diep het bloedsuikergehalte van een konijn of groep konijnen, met een zekere aanvangswaarde, na toediening van een bepaalde dosis insuline moet dalen, indien gegeven is, hoe groot deze daling is in een geval, waarbij men uitging van een andere, bekende, aanvangswaarde. Voor ons heeft dit deze beteekenis, dat wij er ons huidig materiaal aan kunnen toetsen. Gelijk uit de tusschen haakjes geplaatste getallen blijkt, wijken de gevonden gemiddelden op alle rijen, die meer dan 100 waarnemingen omvatten, niet noemenswaard af van de met behulp van den factor van DE LEEUW uit de middelste dier rijen berekende theoretische waarden. Zoowel de nauwkeurigheid der bloedsuikerbepalingen als het aantal waarnemingen waren dus voldoende groot om den regel van DE LEEUW terug te vinden; zij moeten dus ook toereikend zijn geweest om een eventueelen invloed van het lichaamsgewicht op het spoor te komen.

Een blik op de onderste rij leert ons, dat, behalve voor de uiterste, weinig waarnemingen omvattende, groepen, geen sprake is van een geleidelijke toeneming in „bloedsuikersom" van links naar rechts. Dit houdt in, dat bij konijnen met een gewicht van 1900 tot 3000 gram dit gewicht van geen merkbaren invloed was op de reactie op insuline!

Ten einde deze practisch belangrijke conclusie zonder voorbehoud te mogen toepassen bij de ijking, was het noodig aan te toonen, dat zij geldig is *op elk aanvangsbloedsuikerniveau*, m.a.w. dat niet slechts in de onderste rij van tabel III maar ook in elke rij afzonderlijk een climax van links naar rechts ontbreekt. Daartoe moeten de totaalwaarden in elk vakje gedeeld worden door het bijgevoegde aantal waarnemingen, waardoor de verlangde gemiddelden worden verkregen. Het kwam ons voor, dat deze gemiddelden in vele gevallen, door het geringe aantal waarnemingen per vakje, waardeloos waren. Daarom bepalen wij ons in tabel IV (arbitrair) bij de weergave van de gemiddelden tot de vakjes van 20 of meer waarnemingen.

TABEL IV.

Uittreksel van Tabel III voor geselecteerde groepen.

	2050	2150	2250	2350	2450	2550	2650	2750	2850	2950	gemidd.	Aantal	gecorr.
975	2926	2894	2889	2775	2812						2859	(149)	2778
1025	2762	2978	2994	2907	2858	2790	2745	2781			2852	(365)	2844
1075	2855	2975	2942	3014	2933	2927	2810	2925	2926		2923	(476)	2910
1125	3073	3032	3003	2972	2989	2964	3017	2965	2874	2870	2976	(480)	2976
1175			3072	3006	3059	3042	3073	3015	3091	2971	3041	(377)	3042
1225			3204	3188	3169	3132	3038	3147	3013	3052	3118	(241)	3108
gemidd.	2904	2970	3017	2977	2970	2971	2937	2967	2976	2962			
Aantal	(98)	(140)	(265)	(333)	(327)	(296)	(240)	(195)	(111)	(83)		2088	

Het blijkt daarbij, dat inderdaad op geen enkele rij een links-rechts climax bestaat. Ook voor dit (beperkte) materiaal is een „laatste kolom" en een „onderste rij" toegevoegd, waarin de horizontale en verticale gemiddelden van het in de tabel vermelde waarden zijn opgenomen. Daar in *deze* tabel het aantal waarnemingen per vakje reeds in de in die vakjes weergegeven getallen is verrekend, bevatten laatste kolom en onderste rij „gemiddelden van gemiddelden" en dus minder exacte getallen dan de overeenkomstige van tabel III. Desniettemin geeft de laatste kolom (behalve in de bovenste, relatief gegevens-arme, rij) bevredigende overeenstemming met de volgens DE LEEUW berekende theoretische waarden.

In de onderste rij ontbreekt wederom elke neiging tot toeneming der waarden van links naar rechts.

Men kan tenslotte nog de voordeelen van tabel III en IV combineeren met vermijding van beider nadeelen door van de in tabel IV opgenomen vakjes de berekeningswijze van

tabel III toe te passen. Men heeft dan uitsluitend groepen die een behoorlijk aantal waarnemingen omvatten, doch daaruit wordt een *rechtstreeksch* gemiddelde bepaald.

In de plaats van de laatste rij van tabel IV worde dan gelezen: 2891, 2972, 3019, 2969, 2966, 2959, 2939, 2965, 2975 en 2963, en in de plaats van de laatste kolom: 2845 (gec. 2782), 2862 (gec. 2848), 2930 (gec. 2914) 2980, 3042 (gec. 3046) en 3126 (gec. 3112).

Deze getallen geven geen aanleiding tot herziening onzer gevolgtrekkingen.

Eindelijk hebben wij een echte correlatieberekening op ons materiaal toegepast en wel die van LIPS [1]). Op grond daarvan ontbreekt elk verband tusschen lichaamsgewicht en bloedsuikerdaling voor konijnen tusschen 1800 en 3100 gram, te weten ons geheele materiaal, behalve de alleruiterste randgroepen. Dit is dus nog iets meer dan een bevestiging van wat wij na eenvoudige beschouwing van tabel III waagden te besluiten, n.l. geen invloed van het gewicht tusschen 1900 en 3000 gram! Neemt men óók de buitenste, dierarme, groepen erbij, dan levert het materiaal een (geringe) correlatie op.

Het blijft na dit alles de vraag in hoeverre de invloed die het gewicht in de *uiterste* gewichtsgroepen schijnt uit te oefenen reëel is, dan wel voorgewend door de kleinheid van het àantal waarnemingen, dat de gemiddelden weinig betrouwbaar maakt. Ten einde ook deze vraag althans voor de practisch belangrijkere zware konijnen te beslissen, werd opzettelijk aan een vijftal konijnen wier gewicht circa 3.2 kg bedroeg en aan een gelijk groot aantal van circa 2.2 kg dezelfde doses insuline gegeven. Dit werd 4 × verricht op twee verschillende dagen met 0.3 i.E. en 4 × met 0.33 i.E. Tezamen beschikken wij dus over 80 waarnemingen, die een onderlinge vergelijking mogelijk maken. In tabel V wordt van alle gebruikte groepen van 5 konijnen telkens, behalve den proef-datum, het gemiddelde gewicht en de ingespoten dosis, vermeld: het gemiddelde aanvangsbloedsuikergehalte en het gemiddelde *van alle bloedsuikergehalten* ¾, 1½, 2¼ en 3 u *na de injectie.*

TABEL V.

Lichte en zware konijnen met 0.3 en 0.33 i.E. insuline.

Proef	[1941] datum	Groep van 5 kon.	Gemiddeld gewicht kg	Dosis in i. E.	Aanv. bls. mg/l	Gemiddelde bls 3/4—3 u. mg/l
1	28 XI	A	2.28	0.3	1044	746
2		B	2.18		1025	853
3	5 XII	A	2.25		1016	779
4		B	2.19		1054	875
5	18 XII	C	2.10	0.33	1046	766
6		D	2.13		1089	766
7	31 XII	C	2.15		1120	790
8		D	2.18		1120	753
9	28 XI	E	3.29	0.3	1086	733
10		F	3.18		1099	806
11	5 XII	E	3.29		1019	794
12		F	3.18		1089	842
13	18 XII	E	3.21	0.33	1132	767
14		F	3.13		1165	794
15	31 XII	E	3.17		1183	788
16		F	3.10		1169	805

N.B. Het ware te prefereeren geweest, indien de groepen A en B identiek waren geweest aan C en D. Dit was niet het geval. Bepaald hinderlijk voor ons betoog is dit niet.

[1]) Beschreven in WITZIG, Masz und Zahl, 1924.

In de eerste plaats kan uit de tabel worden afgeleid, dat 10 % verschil in insuline zich zoowel bij de lichte als bij de zware konijnen doet gelden in een duidelijk sterkere bloedsuikerdaling.

Vatten wij daartoe eerst de resultaten, verworven aan lichte konijnen (groep 1—8) en dan die aan zware (9—16) tezamen, dan krijgt men de volgende getallen:

		Aanv. bl. 1:	Daling tot gem.:	Na correctie volgens de Leeuw:	
licht	0.3 i.E.	1035	813	813	— 63
	0.33 i.E.	1094	769	750	
zwaar	0.3 i.E.	1073	794	794	— 34
	0.33 i.E.	1162	789	760	

In de tweede plaats kunnen wij tegenover elkaar plaatsen de verschillen tusschen lichte en zware konijnen ingespoten met 0.3 i.E. (1—4, 9—12) en met 0.33 i.E. (5—8, 13—16).

		Aanv. bl 1:	Daling tot gem.:	Na correctie volgens de Leeuw:	
0.3 i.E.	licht	1035	813	813	— 32
	zwaar	1073	794	781	
0.33 i.E.	licht	1094	769	769	— 2
	zwaar	1162	789	767	

Wij zien dan dat de zware konijnen zeker niet zwakker gereageerd hebben dan de lichte. Hun gemiddeld wat hoogere aanvangsbloedsuiker (die overigens na de correctie volgens DE LEEUW geen invloed kan hebben op de proefuitkomsten) was voor een geoefend oog reeds te voorspellen uit de distributie der konijnen over de vakjes van tabel III.

De beschreven voorbeelden uit de practijk der ijking deden ons zien, dat ook voor een gewichtsgroep, waaromtrent onze statistiek ons in het onzekere liet, het gewicht geen beperkenden invloed heeft op de bloedsuikerdaling door insuline.

Beschouwing.

Het moge voor de techniek der ijking gemak opleveren te ervaren, dat het gewicht onzer konijnen binnen wijde grenzen zonder beteekenis is voor den omvang der werking van insuline, niettemin doet het feit ietwat vreemd aan. Men is immers geheel vertrouwd met de ervaring, dat de werking van pharmaca wel niet omgekeerd evenredig is aan, maar toch in hooge mate afhankelijk van het proefdiergewicht. Hoe is het mogelijk, dat in ons geval deze afhankelijkheid ontbreekt? Dit ontbreken zou niet vreemd zijn, indien de gewichtsverschillen schuilen in weefsels, die aan de reactie op insuline nagenoeg niet meedoen, zoodat voor de overige, wèl reageerende, deelen van het lichaam in beide gevallen evenveel insuline ter beschikking staat. Welnu, het ligt voor de hand de gewichtsverschillen tusschen volwassen dieren van één ras grootendeels in het *vet* te projecteeren. Dat het vetweefsel een belangrijke rol speelt in de processen, die door insuline ontketend worden, veronderstelt wel niemand. Zoo beschouwd is aan onze vondst veel van zijn bevreemdend karakter ontnomen.

Overigens overschatte men de draagkracht van onze waarneming niet. Wij vonden niet, dat de omvang van alle reacties van konijnen op insuline onafhankelijk is van hun gewicht, doch slechts dat de, praktisch niet toxische, doseeringen, welke bij de ijking worden gebruikt, een bloedsuikerdaling geven, die niet van het lichaamsgewicht afhankelijk is. Wellicht is met hoogere doses, gemeten aan andere criteria, wel degelijk zulk een effect aantoonbaar. Dit zou inmiddels het belang van onze vondst voor de ijking geenszins verminderen.

Summary.

On the ground of 2593 data obtained from rabbits, it was shown that after low doses of insulin there is no correlation between the bloodsugar decrease and the bodyweight fluctuating between 2—3.2 kg. This conclusion is confirmed by recent experiments, in which heavy and light rabbits were injected with different doses of insulin. It is not necessary when standardizing insulin with the aid of chinchilla rabbits to maintain the division of rabbits into two groups of animals with equal weight, provided the weights are between 2 and 3.2 kg as mentioned.

Medicine. — *Anatomical and experimental investigations about the muscular system of the mammalian lung.* By C. VERSTEEGH (Otolaryngologist) and C. DIJKSTRA (Physician f. lung diseases). (From the physiological Laboratory of the University, Utrecht, director the late Prof. A. K. M. NOYONS.) Communicated by Prof. A. DE KLEYN.)

(Communicated at the meeting of September 26, 1942.)

PART II.

2nd Experiment.

A second possibility of investigation offered by this experiment, is the following. In turning off simultaneously both the outlet and the supply of the system, in the whole system, and therefore also in the lungs, a certain quantity of oxygen, under slight over-pressure (2—4 cm H_2O) is present. By keeping both the heart action and the lung circulation going, the interchange of gas in the lungs will remain, i.e. oxygen will pass from the lungs into the blood and CO_2 will be excreted into the lung. Whether this interchange of gas causes an alteration of the pressure of the now locked system is dependent on the mutual relations between both gasses O_2 and CO_2; if per time unit more O_2 is absorbed than CO_2 excreted, in other words, when a low respiratory quotient exists during the experiment, a fall of the pressure in the system must occur.

This appears to be indeed the case (see Fig. 8).

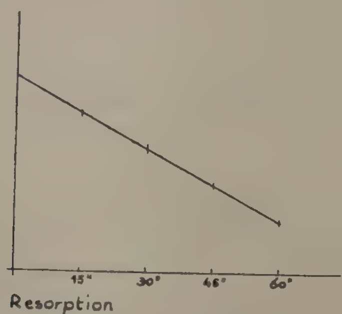
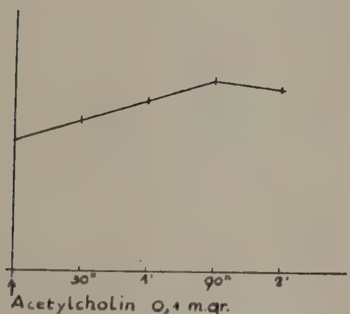


Fig. 8. Decrease of pressure in the so-called "closed system". The supply- and outlet-tube of the lungs are simultaneously closed. The pressure in the lungs decreases by the oxygen resorption in the closed system (which is greater than the excretion of CO_2) as can be seen from this "resorption-curve".

When, during this so-called "resorption-test" the lung musculature is made to contract, the volume in the system will diminish, thus causing an increase of the pressure. Consequently the fall of pressure, due to the resorption of O_2 will be counteracted more or less. It must, however, be borne in mind, that a small fall of pressure in the closed system can also occur after an alteration of the circulation. So a less marked fall furnishes no evidence of a contraction in the lung musculature. Only when, during the resorption test, an increase instead of a decrease of the pressure in the system develops, it is proved that a diminution of the volume of the lung has been effected.

From the following curves (Fig. 9 and 10) it appears that both acetylcholine and histamine cause a distinct rise of the pressure in the system. A drawback of this experi-

ment, however, is the fact, that it is impossible to judge if and to what extent the air volume in the lung is diminished by alterations in the quantity of blood in the lungs (resp. by the presence of pulmonary oedema). This can also cause an increased pressure in the closed system. Moreover, the contraction of the bronchial musculature, causing a stricture of the bronchi, will influence the total volume of the system. So from a registration — if made —, of an increased pressure, it cannot be concluded with certainty that a diminution of the lung volume, due to a contraction of the peripheral lung musculature, is present.



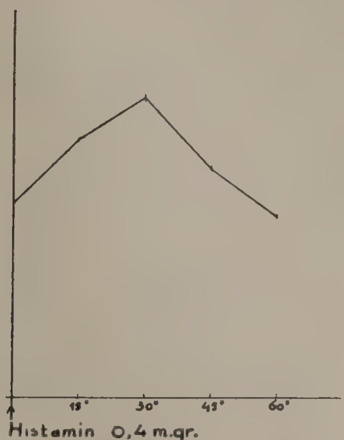


Fig. 9 and 10. Increase of pressure in the *closed* system. The decrease of pressure due to the O_2 resorption during this experiment is overcompensated by the diminution of the pulmonary volume caused by the muscular contractions under influence of acetylcholine (0.1 mgm), resp. histamine (0.4 mgm), so that in the system an increased pressure occurs.

3rd Experiment.

Finally a third series of experiments was made. In the tank R. oxygen is present under rather high pressure (¼ atmosphere). Opening of the tap will cause a flow of this oxygen from the tank to the system. As the gas in the tank is under rather high pressure, the pressure in the system increases suddenly during a short time. Because the gas can escape at *U*, the pressure in the system drops after some time to the original level. This temporarily present rise of pressure is dependent on the resistance offered by the lung to the quantity of gas that is flowing in. In proportion as the lung is more rigid, its distention will be smaller under this influence. Consequently the result is only a small increase of the pulmonary volume, in other words, the pressure increases relatively more. The reverse, i.e. a more marked dilatation of the lung (a less marked increase of pressure) will take place when the lung is non-rigid.

Administration of histamine and acetylcholine enabled us to demonstrate a distinct alteration of the resistance offered by the lungs to the gas which is flowing in . (Fig. 11 and 12.)

So the degree of this sudden increase of pressure is, to a certain extent, a standard for the rigidity of the lungs. This rigidity is, among other things, dependent on the tonus of the peripheral pulmonary muscles; increase of the tonus of these muscles will cause a relatively sharp rise of the pressure. However, the rigidity of this organ is also undoubtedly influenced by a changed blood filling of the lungs (resp. by formation of oedema) and these factors cannot be excluded from the experiment with certainty. Moreover, the diameter of the bronchi will have some influence upon the result of the experi-

ment. If the bronchial system is narrowed by acetylcholine or histamine, this will cause a larger resistance to the flow of the oxygen, in other words, the rise of pressure in the system will increase, causing the same effect as an increased pulmonary rigidity. As it is impossible to give a further analysis of the different factors which play a part in this experiment, we left it and passed on to the following experiment.

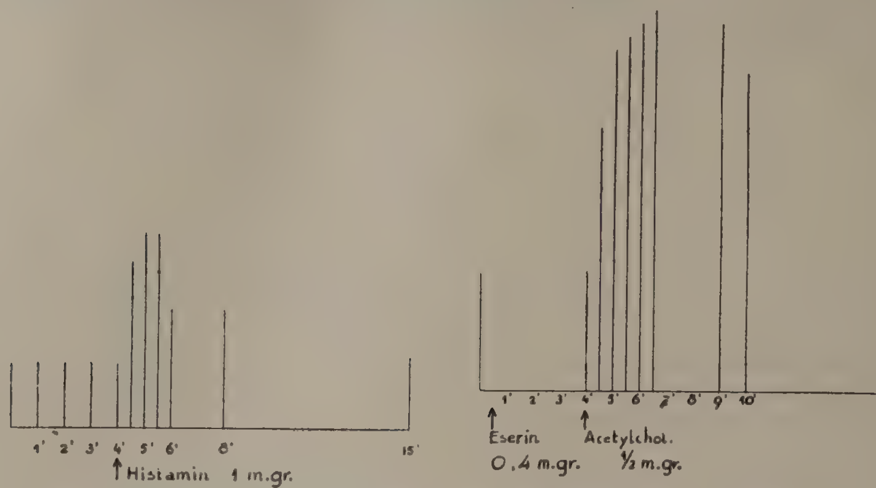


Fig. 11 and 12. Increase of pressure in the *open* system due to an inflow of ± 50 cc O_2 under a pressure of ± ¼ atmosphere. At ↑ injection of histamine (1 mgm), resp. acetylcholine (½ mgm). The resistance offered by the lung to the gas that flows in (expressed in an increased pressure in the system) is markedly greater than before the injection.

4th Experiment (Fig. 13).

The experimental animal, operated upon the same manner as in experiment 1, is in the closed space. In the glazed wall of this space there are three openings, as can be seen from Fig. 13. The first opening is connected with the trachea, the second is used for the supply of a cannula in the vena jugularis, and the third for the registration of the atmospheric pressure in the space in which the animal is placed. This pressure is registered by a manometer, the variations in pressure on a kymographion by a MAREYSCH' tambour. In the closed space a slight negative pressure (2 cm H_2O) is made and maintained. By this the lungs, which lie completely free, will somewhat unfold themselves.

The air in the lungs combines constantly with the atmospherical air via the tracheal cannula and the opening *U*. To keep the animal alive artificial respiration must be applied. This is done by means of a pump, which regularly pumps in certain quantities of air with a frequency of 40—50 beats a minute. This air partly enters the lungs via I and the tracheal cannula and escapes partly at *U*. Each pump-stroke with following insufflation of the lungs, will compress the air in the closed space in which the experimental animal is placed, which is expressed in the registration of the manometer-variations. In each interval between the pump-strokes, the lungs will collapse again by the elasticity of the lung tissue. For this a certain space of time is necessary; if the intervals between the pump-strokes are large enough, the lungs will each time regain their original volume and the pressure in the closed space will return to its original value. The registration of the pressure in the closed space takes place in such a way that, with each pump-stroke, causing an increased pressure, the pointer on the kymographion goes upwards and returns to its original state during the interval.

So in taking care that the intervals between the pump-strokes are large enough, the

pointer will fall back at every turn to the original state, corresponding with a pressure of —2 cm H_2O. This fact can be checked by stopping the pump-installation during the experiment. It then appears that the feet of the curve are at the same height (see Fig. 14).

In studying the function of the involuntary lung muscles we are dealing with two groups of muscles. First the bronchial muscles which influence the lumen of the bronchi, second the peripheral lung musculature of which the function will be examined by us and of which it can be expected that it will reduce the lungs by contraction. Alterations of the tonus of both these muscle-systems will, when elicited separately, each exercise a particular influence upon the lungs which is expressed on the curve via the pressure in the closed space.

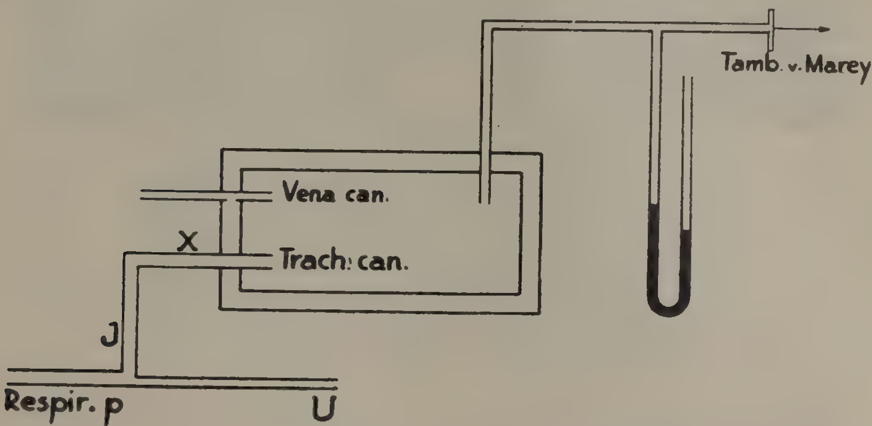


Fig. 13. The experimental animal is placed in a completely closed space, which communicates with the atmospherical air only by means of a tracheal cannula and a venae cannula. The pressure in the space is measured by a manometer; the variations in pressure in the manometer are registered on a kymographion by a MAREYSCH' tambour. The lungs are ventilated artifically by a respiration pump. The thorax of the animal is opened widely. A slight negative pressure (2 cm H_2O) is made and constantly kept in the closed space.

Contraction of the peripheral lung musculature will reduce the pulmonary volume; moreover, owing to an increased resistance of the lungs, less air will be inflated into the lungs at each pump-stroke. *This is expressed in the curve by a decline of the feet* (below the line of 2 cm H_2O), combined with a diminution of the records which are caused by the artificial respiration. An isolated bronchial stricture gives another effect and can be imitated by the following experiment. The tracheal cannula is narrowed at *X* which causes a similar effect as given by an isolated bronchial stricture.

From Fig. 15 it appears that the main effect consists of a diminution of the variations in pressure which are caused by the artificial respiration. This can be understood very well as both the air that flows in and the air that flows out, can pass the stricture less easily. Besides, as can be seen from the curve, the feet do not fall to the original height. From this it appears that the lungs are unable to drive out the air which is inflated each time by the pump, before the next pump-stroke; so the volume in expiration-position has increased by the stenosis-respiration.

Taking the obtained curves as a basis we shall be able to distinguish the contractions of the bronchial muscles from those of the peripheral lung musculature, because the *bronchial stricture* causes a diminution of the variations of the pulmonary volume, *by which the feet of the curve go upwards; contraction of the peripheral lung musculature*

also gives rise to smaller variations of the lung volume, *but now the line, connecting the feet, falls, as the lung volume diminishes.*

This of course only holds good when the alterations occur separately or when one effect is distinctly stronger than the other. However, nature does not make things so easy.

Fig. 14. Curve showing the variations in pressure in the closed space caused by the artificial respiration. The variations in pressure are absolutely constant; the feet of the curve form a straight line. If the respiration-pump is stopped, the lungs collapse; the pressure in the closed space is similar to the pressure at the end of the expiration during the artificial respiration.

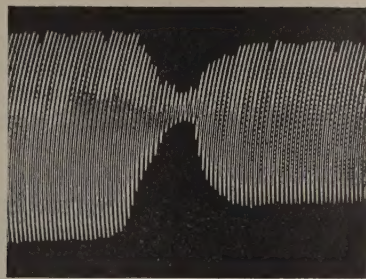
Fig. 15. Curve showing the variations in pressure in the closed space caused by the artificial respiration. At ↑ the tracheal cannula is artificially narrowed. By this the quantity of air per respiration is diminished (smaller result on the curve). The feet of the curve are now on a higher level than at the beginning of the curve, i.e. the lung volume (at the end of the expiration) has increased during the presence of the stenosis in the trachea. As soon as the stenosis is removed the normal condition reappears.

We may expect that, after injection of a certain pharmacum, as a rule a combined influence, i.e. in both muscular facisculi, will develop. The influence of this combined effect upon the curve cannot be determined beforehand, however. For the different factors as latency, strength of the tonus alteration of the different muscular fasciculi, influence of possible antagonistic activities etc. are not sufficiently known and incalculable, moreover.

Instead of risking further theoretical considerations, we had better study, by means of experiments, the influence of different pharmaca upon the lung volume and upon the respiratory movements.

In curve 16 and 17 the so-called "bronchial effect" of acetylcholine is clearly demonstrated. In curve 16 a passage through the bronchi is still possible which appears from the variations in pressure which are still present during the artificial respiration. In the second case (Fig. 17) the bronchial stricture is so marked that we can almost speak of an enclosure of the bronchi; the variations in pressure caused by the artificial respiration can scarcely be observed. At ↑↑ the pressure in the trachea is increased artificially, by which one succeeds again more or less in making a passage through the bronchi possible; the variations in pressure, however, remain smaller than before the influence of the acetylcholine, so that it appears that the acetylcholine still has some influence upon the bronchial muscles. In both cases the pulmonary volume at the end of the respiration, during the acetylcholine activity is larger than before. Both these curves show essentially the same thing as Fig. 15, where, by mechanic enclosure of the trachea, a similar effect was obtained. In the above mentioned cases an influence upon the peripheral lung mus-

culature (which would cause a diminution of the lung volume) could not be perceived; the effect upon the bronchi dominates.



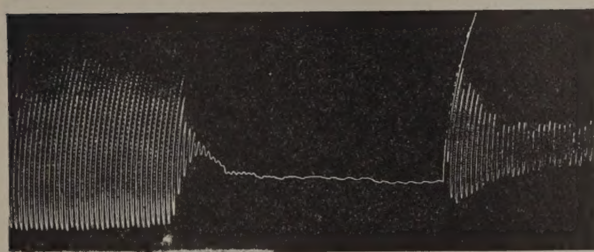


Fig. 16 and 17. Curves showing the variations in pressure in the closed space during the artificial respiration. In both cases, after treatment with 2 cc. eserine 1/5000, 0.2 cc acetylcholine (Roche) 1/1000 is injected in the vena. A marked stricture of the bronchus occurs which, in the second case (curve 17) is so strong that a complete enclosure of the bronchial lumen occurs. At ↑↑ it was tried to unfold the lungs by means of an increase of the intratracheal pressure and to open the bronchi. In this we only partly succeeded, however.

From *curve 18* it appears that the acetylcholine activity can also cause a diminution of the pulmonary volume. Here, after injection of acetylcholine, the feet of the curve go distinctly downwards i.e. the pulmonary volume at the end of the respiratory movement has become smaller. It also appears that the variations in pressure have decreased during respiration. This can be caused both by an increased tonus (resp. contraction) of the peripheral lung musculature and by a contraction of the bronchial muscles.

We know however that in bronchial stricture the lung volume becomes greater, during

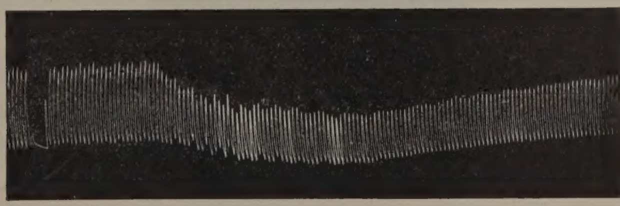


Fig. 18. Diminution of the lung volume under influence of 0.2 cc 1/1000 acetylcholine. The negative pressure in the closed space increases, which can be seen from the fall of the feet in the curve; i.o.w. the pulmonary volume has diminished. At the same time the variations in pressure due to the artificial respiration have become smaller.

expiration. If we accept, that the injection of acetylcholine in Fig. 18 has influenced the bronchial musculature and thus caused the diminuation of the respiratory movement, so the fact that in this case the pulmonary volume becomes smaller is still of more importance. The diminuation of the lung volume caused by the construction of the peripheral lung musculature should be in reality greater than appears from the curve.

From the different experiments we learned that the same dosage of pharmaca (acetylcholine) gave different effects. Sometimes a bronchial effect occurs, in other cases the reactions of the peripheral lung musculature are prominent. Repeatedly the same dosis gave no effect at all.

Fig. 19. Influence of histamine, 0.2 cc 1/1000 upon the lung volume and the bronchi (see for further explanation also fig. 18).

The influence of histamine upon the lung musculature can be seen from *Fig. 19*. This curve gives the same picture as Fig. 18. Histamine also, although not always, causes a diminution of the lung volume. The fall of the feet of the curve is, in this case, more marked than in Fig. 18, but otherwise both curves resemble each other much. To obtain an impression of the diminution of the lung volume in this case at the end of the experiment 20 cc of air was sucked from the closed space by a record syringe and then again injected. It now appears (see *Fig. 20*) that the fall in curve 19 nearly corresponds to the

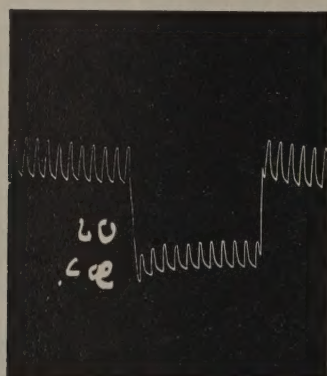


Fig. 20. Gauge-curve. A diminution of the volume in the closed space of 20 cc brings about a decrease in the curve which is shown in this figure. The difference in height of the feet of curve 19 (before and during the histamine-activity) is about the same as that in fig. 20.

fall obtained by the sucking out of 20 cc of air. So the diminution of the lung volume caused by the histamine activity was about 20 cubic cm and could even be observed with the naked eye.

It must, however, be emphasized that there is not any regularity in our results. The effects caused by one and the same dosis of acetylcholine can be quite unlike. Sometimes

a bronchial stricture occurs, in other cases the contraction of the peripheral lung musculature is predominant, but most frequently the first influence is found. Histamine, as a rule influencing the peripheral lung musculature, frequently has a magnificent influence, whereas at other times the same dosage has no effect at all. What can be the cause of this irregularity and these different reactions of the experimental animals, has not become clear. In our opinion also in this experiment the possibility cannot be excluded that a changed filling of the vessels, respectively the development of lung oedema, has influenced the result of the experiment, so that here too several factors have played a part.

Taking curve 19 as an example we cannot believe, however, that a changed filling of the vessels in the lung or lung oedema has caused the effect; the diminution of the volume of the air in the lungs therefore is too large and takes too little time. Some minutes after the injection the effect had already disappeared, which would not have been the case in lung oedema. *However it may be, from our experiments it appeared that a diminution of the lung volume, due to the influence of acetylcholine and histamine can develop, which, in our opinion, is mainly due to a contraction of the lung musculature.*

Not only in pathology (BRONKHORST) but also in physiology we shall have to take into account reactions of this peripheral lung musculature. In this connection the investigations of VERZAR must be mentioned. He found that a dilatation of the lung volume occurs after muscle activity or a sojourn in the mountains, which symptoms he considers, next to acceleration and deepening of the respiration, as "dritter Form der Atmungsregulation". He thinks that the peripheral lung musculature plays a part in this regulation of the pulmonary volume. By way of an increased secretion of adrenalin in these cases a tonus-diminution of the fasciculi would develop, by which the thorax dilates by reflex.

In our experiments, however, no marked influence of adrenalin could be observed.

REFERENCES.

1. BALTISBERGER, Ztschr. Anata., **61** (1921).
2. BINET en BURSTEIN, Presse médicale, **47** (1939).
3. BRONKHORST und DIJKSTRA, Beitr. z. Klin. Tbk., **94** (1940).
4. CARLSON and LUCKHARDT, Am. Jnl. physiology, **54** (1920), **55** (1921).
5. DIJKSTRA et NOYONS, Arch. internat. Phys., **49** (1939).
6. ELLIS, Lancet (1938), p. 819.
7. MACKLIN, Physiol. Rev., **9** (1929).
8. NEERGAARD, Ztschr. exp. Med., **66** (1929).
9. VERZAR, Schw. med. Wochenschr., **63** (1933).

P. 1459/1.

Verantwoordelijk voor den geheelen inhoud: M. W. WOERDEMAN te Amsterdam. Uitgever: N.V. Noord-Hollandsche Uitgevers Maatschappij, N.Z. Voorburgwal 68—70, Amsterdam. Drukker: Drukkerij Holland N.V., N.Z. Voorburgwal 68—70, Amsterdam.